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TO NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

RADIOISOTOPIC BIOCHEMICAL PROBE FOR EXTRATERRESTRIAL LIFE

(NASA Contract No. NASr-10)

2

Gilbert V. Levin et al

September 25, 1963

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TO

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

RADIOISOTOPIC BIOCHEMICAL PROBE FOR EXTRATERRESTRIAL LIFE

CONTRACT NO. NASr-10



September 25, 1963

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## I. SUMMARY

Pure cultures and microorganisms in soils have continued to yield positive responses when cultured in labelled M8 medium. Three pure cultures and five soils have been added to the test collection. Nitrobacter agilis; Serratia marcescens; Ferrobacillus ferrooxidans; a field soil from Rutgers University Agriculture School; a forest soil from Metuchen, New Jersey; a desert soil from Apple Valley, California; a mountain-tundra soil from the Rocky Mountains; and a salt soil from the San Francisco Bay area in California. A general screening for colony types carried out on the new soils yielded an estimated 10-15 different bacteria, 5-10 different streptomycetes, and 10-15 different fungi. A recheck of the metabolically active Soil Isolate D, isolated from a field soil and identified as a streptomycete, verified the results obtained in the preceeding quarter. Rhodospirillum rubrum grown anaerobically and photosynthetically, produced positive results. An anaerobic determination performed on segments of soil-inoculated collecting line removed from the culture chamber of Gulliver also yielded a positive response.

The study of acrolein for use as an antimetabolite showed it to be heat stable and chemically unreactive with the labelled substrates, but not completely effective as an inhibitor in the concentration employed. Evaluation will continue, using the compound at higher concentrations.

A preliminary photosynthetic determination was carried out with the alga Scenedesmus quadricauda. The study will be completed in the next quarter.

Five field tests were performed with Gulliver, and all yielded positive responses. The Bard Parker antimetabolite continued to be effective. As a result of the premature saturation of the C<sup>14</sup>O<sub>2</sub> collectors, an intensified program is in progress to rectify the problem.

Duane G. Hoffman has joined the staff and project as Design Engineer, and will be responsible for quality control procedures. Conferences pertaining to the Gulliver experiment were held with personnel from the Jet Propulsion Laboratory and the Ames Research Center. A paper on the Gulliver space probe was presented at the COSPAR Fourth International Space Sciences Symposium, Warsaw, Poland.

## II. BIOLOGICAL INVESTIGATION

The capacity of the automated monitoring and recording system has been increased from 8 to 12 units, thereby allowing twelve simultaneous comparisons to be made. In addition, an automatic sample changer and recorder have been provided for the gas flow counter.

### A. RESPONSES OF TEST MICROORGANISMS

#### 1. KNOWN CULTURES

Three pure cultures of soil microorganisms have been added to the test collection - Nitrobacter agilis (ATCC 14123), Serratia marcescens,\* and Ferrobacillus ferrooxidans\*\*. Pertinent Characteristics of each are given below:

- (1) Nitrobacter agilis - aerobic, autotrophic, non-spore former, non motile, oxidizes nitrites, gram negative rod
- (2) Serratia marcescens - facultative aerobe, heterotrophic, non-spore former, motile, gram negative rod
- (3) Ferrobacillus ferrooxidans - aerobic, autotrophic, motile, oxidizes ferrous iron to ferric iron, gram negative rod.

The Nitrobacter agilis and Ferrobacillus ferrooxidans are presently being subcultured and have not yet been tested. The Serratia marcescens was used as the inoculum in a comparison of glucose of various specific activities. The response, obtained on the automatic monitoring unit, was rapid and high;  $1.3 \times 10^7$  cells produced an average of 18,750 cpm within half an hour in M8 medium containing formate (0.13 mM, 3.3 uc/ml) - glucose (0.21 mM, 1.0 uc/ml) - lactate (0.44 mM, 2.2 uc/ml).

Bacillus subtilis var globigii (as vegative cells), Escherichia coli, Rhodospirillum rubrum, and Saccharomyces cerevisiae were used routinely in various experiments. Positive responses were obtained in all determinations. Actual responses are reported in later sections.

\* Obtained from Mrs. B. H. Caminita, Bacteriologist U. S. Naval Weapons Laboratory, Dahlgren, Virginia

\*\* Obtained from Dr. Henry L. Ehrlich, Assoc. Prof. of Biology  
Rensselaer Polytechnic Institute,  
Troy, New York.

## 2. SOILS

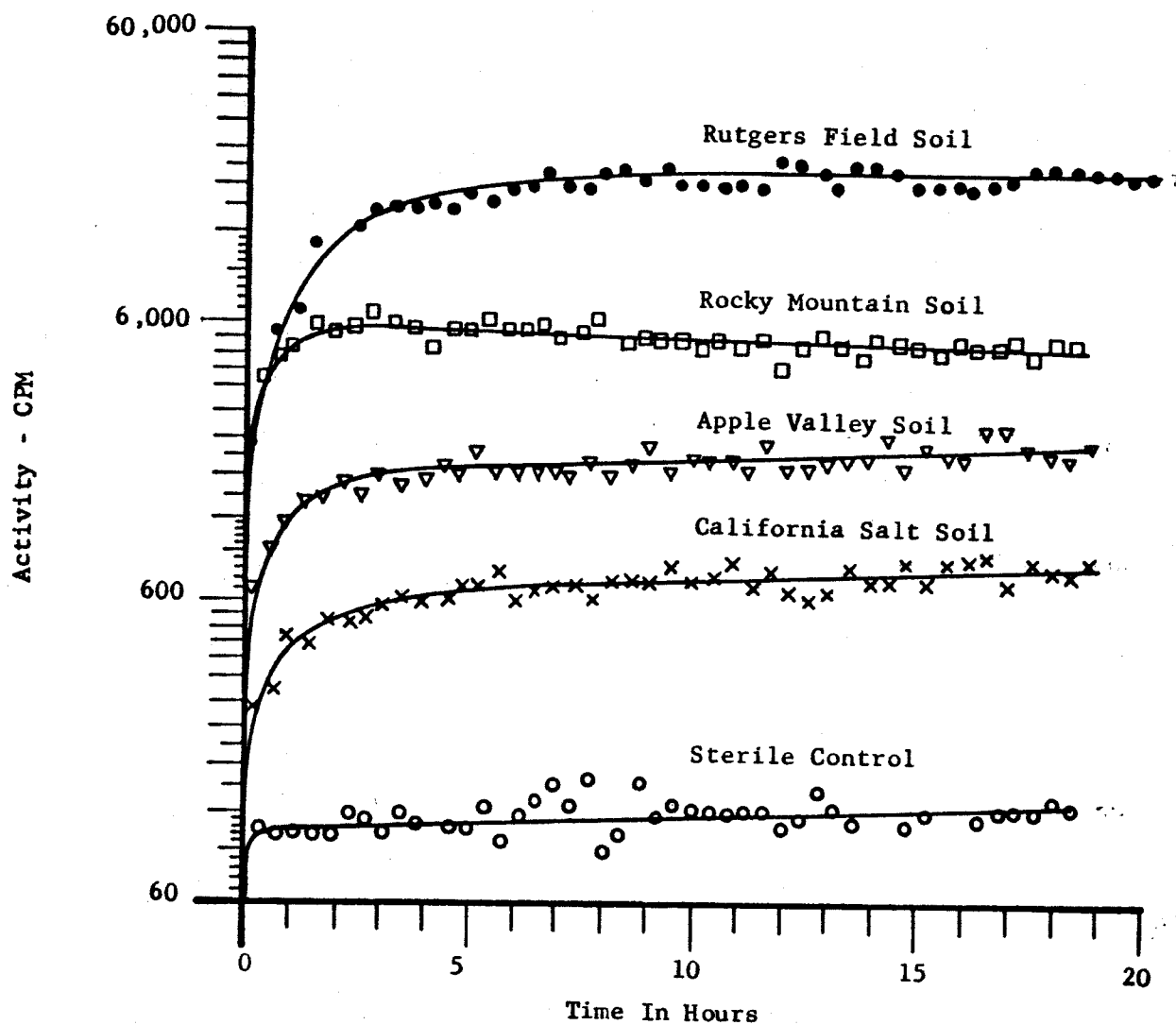
The study and testing of soils has continued to comprise a major portion of the biological program. The soils have been used routinely as inocula for various laboratory determinations. All soils tested, many coming from widely varying environments, have responded rapidly. The possibility exists that these responses are caused by a narrow spectrum of species which might be fairly common to most soils. Other species peculiar to the respective soils might constitute the majority of the total species and yet respond poorly. It is important, therefore, to determine whether a true general response is being obtained from the many species present.

In the previous quarter, soils were plated for isolation of individual microbial genera. Six predominant isolates were obtained and tested to determine if any correlation existed between the individual isolate response and total response of the same soil. To identify the genus and species of each isolate from each soil would be an extensive and time consuming project. Therefore, a more general type of screening program has been initiated. All soils will be routinely tested in the M8 medium and plate counts made to determine cell numbers. Colonies will be described and gram stained. The colonies appearing most frequently throughout the various soils will be isolated and tested for similarity and intensity of response.

The general screening procedure is being carried out with soils recently added to the test collection. They include a field soil from Rutgers University Agriculture School, a forest soil from Metuchen, New Jersey, a desert soil from Apple Valley, California, a mountain-tundra soil from the 12,000 foot level of the Nevada Rocky Mountains, and a salt soil from the San Francisco Bay area, California. All the soils tested yielded positive responses in the M8 medium. Figure 1 depicts typical responses from four of the soils. The Metuchen-forest soil is presently being tested. The results of the plate counts are given in Table 1.

FIGURE 1

RESPONSES FROM SOILS TO M8 MEDIUM



Medium: 0.2 ml/test

Inoculum: 100 mg Soil

Formate-C<sup>14</sup> 0.13 mM  
 Glucose-C<sup>14</sup> 0.14 mM  
 Lactate-C<sup>14</sup> 0.44 mM

3.30 uc/ml  
 0.66 uc/ml  
 2.20 uc/ml

Table 1  
NUMBERS OF ORGANISMS ISOLATED FROM TEST SOILS

Soil		Colonies/100 mg	
<u>Type</u>	<u>Location</u>	<u>Tryptone-glucose agar</u>	<u>Sabouraud agar</u>
Desert	Apple Valley, California	49,000	50
Mountain	Rocky Mountain, Colorado	5,950,000	86,000
Field	Rutgers, New Jersey	608,000	6,750
Forest	Metuchen, New Jersey	81,000	20,500
Salt	San Francisco Bay, California	1,700	0

The salt soil was also plated on a halophilic agar containing 20 percent NaCl. No growth appeared. The soil is presently being cultured in media of lesser salt concentrations in an effort to isolate specific halophilic organisms.

The colony screening on four of the soils yielded an estimated total of 10-15 different bacteria, 5-10 different streptomycetes, and 10-15 different fungi. Pure cultures of these organisms are being prepared for gram staining and further comparative study.

### 3. SENSITIVITY

A recheck of Soil Isolate D, the streptomycete isolated from a field soil, first reported in Progress Report No 9, proved it to be quite active metabolically. In the experiment previously reported M8 medium with 1.00 mM of formate and 0.33 mM of glucose was used. In the present determination, the isolate was used as an inoculum in an investigation of the effects of various molar concentrations of the formate-glucose C<sup>14</sup> combinations. The recheck was done in M8 medium containing 0.40 mM formate and 0.40 mM glucose. The decreased molar level may account for the slightly lower response. The two determinations are compared in Table 2.

### 4. ANAEROBIC DETERMINATIONS

The difficulty of achieving anaerobic conditions in the laboratory test chamber has delayed the planned studies with anaerobic organisms. However, additional laboratory apparatus has been designed specifically to permit automated determinations. When complete, prime emphasis will be placed on this phase of the investigation. Two anaerobic experiments were performed utilizing the Brewer Jar. In the first, Rhodospirillum rubrum was grown photosynthetically under anaerobic and aerobic conditions for comparison. After four hours of incubation in planchets and 15 minutes of C<sup>14</sup>O<sub>2</sub> collection with wet Ba(OH)<sub>2</sub>,  $2.5 \times 10^5$  cells produced an average of 917 cpm aerobically, and 300 cpm anaerobically. In the second experiment, in an effort to determine the presence or absence of anaerobic microorganisms in the June 19

Table 2

COMPARISON OF THE METABOLIC ACTIVITY OF SOILISOLATE D - A STREPTOMYCETERadioactivity - CPM Above Sterile ControlTime in Hours

<u>Date</u>	<u>Cells/Test</u>	<u>Medium</u>	<u>0.5</u>	<u>1.0</u>	<u>2.0</u>	<u>4.0</u>
June, 1963	118	M8 1.00 mM formate	75	345	590	1,290
	177	0.33 mM glucose	165	465	585	1,590
August, 1963	1,680	M8 0.44 mM formate	210	273	398	580
		0.44 mM glucose				

field test, anaerobic determinations were performed on segments of the soil-inoculated collecting line. Positive results were obtained which suggested that anaerobes were present in large numbers. This is discussed fully in Section II, Field Tests.

Gulliver has thus detected anaerobic organisms under anaerobic conditions. However, the generic spectrum of anaerobes which can be detected has yet to be established.

#### B. LABELLED SUBSTRATE CONCENTRATION

The investigation of labelled substrates during the past quarter has been concerned primarily with the determination of molar levels for the comparative study of new labelled substrates.

Experiments were conducted on the automated monitoring unit to determine the effect of alterations in concentration and radio-activity levels. Appropriately labelled M8 was inoculated with Bacillus subtilis var globigii, Escherichia coli, Saccharomyces cerevisiae, Soil Isolate D, salt soil, and mountain soil. The following C<sup>14</sup> combinations were incorporated:

- |     |                                |   |
|-----|--------------------------------|---|
| (1) | formate - 5.00 uc/ml, 0.20 mM  | } Previously reported<br>(Quarterly #9) |
|     | glucose - 1.00 uc/ml, 0.20 mM  |   |
| (2) | formate - 10.00 uc/ml, 0.40 mM | } Two fold increase<br>over (1)         |
|     | glucose - 2.00 uc/ml, 0.40 mM  |   |
| (3) | formate - 6.50 uc/ml, 0.26 mM  | } Molarity equal<br>to (2)              |
|     | glucose - 1.30 uc/ml, 0.28 mM  |   |
|     | lactate - 1.30 uc/ml, 0.26 mM  |   |

No conclusive results have, as yet, been obtained. This study is being continued.

#### C. EFFECTS OF INCREASED SPECIFIC ACTIVITY

Glucose-C<sup>14</sup>, a major radioactive constituent of the basal medium, was investigated to determine the effect of increased specific activity. The study of formate-C<sup>14</sup> (Quarterly Progress Report No.9) showed that a higher specific activity (25 mc/mM) and a lower concentration (0.20 mM) produced the most advantageous response.

The glucose determination was conducted in the following manner - glucose having a specific activity of 4.73 mc/mM was compared with glucose having a specific activity of 12.00 mc/mM on a molar and radioactive basis. (During the past year the specific activity of glucose purchased varied, 3.00 mc/mM or 4.73 mc/mM). Formate and lactate were incorporated at constant levels. The combinations tested are shown in Table 3. The automated monitoring unit was used. One-tenth ml of an appropriately diluted broth culture of Escherichia coli, Rhodospirillum rubrum (aerobically, non-photosynthetically), Saccharomyces cerevisiae and Serratia marcescens, respectively, was inoculated into 0.5 ml of the tagged M8. When garden soil was used as the inoculum, 0.2 ml of the medium were seeded with 100 mg of the soil. Sterile controls were employed throughout. The following results were obtained:

- (1) in the formate-glucose combinations all responses were very similar, although the 4.73 mc/mM - 0.20 mM combination (Table 3) showed a very slight advantage
- (2) in the formate-glucose-lactate combinations (Table 3) all responses, again, were similar.

It appears that the use of an increased specific activity of glucose would offer no advantage if the concentration is decreased when used with formate in the basic radioactive substrate standard or with formate and lactate in the triple-tagged medium. As stated above, concentration effects will be investigated further.

#### D. ANTIMETABOLITES

The search for a more efficient antimetabolite has continued with the evaluation of acrolein ( $\text{CH}_2=\text{CHCHO}$ ).

The investigation followed the pattern of the original screening program for inhibitors. The acrolein was sterilized at 135°C for 26 hours and used in a final concentration of 20 ppm. Two sets of planchets were inoculated with selected organisms, one set contained the antimetabolite, the other was uninhibited. Sterile controls

Table 3

COMBINATIONS TESTED FOR EFFECTS OF INCREASED SPECIFIC ACTIVITY OF GLUCOSE C<sup>14</sup>

Combination	Specific Activity-mc/mM	Molarity-mM	Radioactivity-uc/ml	Total Activity uc/ml
Formate Glucose	25.00 4.73	0.20 0.20	5.00 1.00	6.00
Formate Glucose	25.00 12.00	0.20 0.08	5.00 1.00	6.00
Formate Glucose	25.00 12.00	0.20 0.20	5.00 2.50	7.50
Formate Glucose Lactate	25.00 4.73 5.00	0.13 0.14 0.44	3.30 0.66 2.20	6.16
Formate Glucose Lactate	25.00 4.73 5.00	0.13 0.21 0.44	3.30 1.00 2.20	6.50
Formate Glucose Lactate	25.00 12.00 5.00	0.13 0.21 0.44	3.30 2.50 2.20	8.00

accompanied each set. Following two hours and 3.5 hours of incubation,  $C^{14}O_2$  was collected for 15 minutes with wet  $Ba(OH)_2$  pads. The collection pads were dried and assayed in the gas-flow counter. Only Saccharomyces cerevisiae was completely inhibited. Bacillus subtilis var globigii, Escherichia coli, and the organisms in a mountain soil were only partially inhibited.

Because of the unfavorable results, an additional tube turbidity test was performed on two of the organisms, Escherichia coli, and Saccharomyces cerevisiae, with acrolein that had not been sterilized and acrolein which had been sterilized. It was possible that the potency of the inhibitor was affected by the sterilization process. The non sterile acrolein, at the same concentration, was placed in one set of inoculated test tubes - the second inoculated set was uninhibited. A duplicate experiment was run with the sterilized acrolein. At the end of 24 hours, heavy growth was evident in the non-inhibited tubes with a partial inhibition in the tubes containing the sterilized and non-sterilized acrolein. The indications are that the sterilization had little influence upon the effectiveness of the compound. Despite the poor inhibition responses obtained with the acrolein in this study (it did meet the requirements of heat stability and unreactiveness with labelled substrate) it has been claimed as an effective antimetabolite in other laboratories. Further investigation is merited and the study will be continued in the forthcoming quarter.

#### E. PHOTOSYNTHESIS

The light-dark response previously reported was studied in a preliminary manner using a different genus of algae. Chlorella pyrenoidosa was used before; this quarter Scenedesmus quadricauda was used as inoculum. Sodium lactate- $C^{14}$  was used as the labelled substrate in a basal urea medium. Again a positive light-dark response was obtained, but as yet, the

experiment has not been repeated often enough to permit definitive conclusions. This will be continued and results available for the next report.

#### F. FIELD TESTS

Field testing of Gulliver III has continued. Information and data pertinent to mechanical reliability (Section III Instrumentation) and response sensitivity has been augmented by five additional tests - conducted, programmed, and monitored in accordance with previous Model III operative procedures. The field testing program has continued to yield positive and rapid biological responses, effective antimetabolite results, and comparable sterile control levels. It has also emphasized the need for continued extensive research with the  $C^{14}O_2$  collectors.

Various degrees of collector saturation were encountered in all tests. Although the responses obtained were rapid and generally satisfactory, they were lower than desired, having been partially masked by the saturated collectors. Efforts are being extended to rectify this problem.

The use of the basal M8 as a field testing medium has continued. The radioactive substrates, formate-glucose-lactate, were incorporated at the levels shown in each of the field test figures. The altered  $C^{14}$  concentrations were the result of the continuous laboratory efforts in medium improvement.

The Bard Parker inhibitor was employed in four of the tests. In two of the four, the antimetabolite was injected after the count rate reached 500 cpm and 1,000 cpm, respectively. In the other two, it was injected immediately following the breakage of the medium ampoule, thus allowing the inhibitor to be dispersed by the revolving retrieval motor. In all four tests the antimetabolite was effective - a definite difference was apparent between the uninhibited and inhibited chambers. When the Bard Parker was injected immediately, this difference appeared earlier,

was more pronounced, and remained at a level corresponding to the sterile control. (The sterile control level for Model III, incorporating the field test medium in present use, was determined by the June 4th field test, Figure 2). The Bard Parker will continue to be used until further experimentation produces a more desirable inhibitor.

The June 19th field test (Figure 3) resulted in a lower response than usual. In previous tests the baffle pin was removed from the instrument allowing oxygen to enter the culture chamber. The resultant atmosphere favored the aerobic microorganisms in the surface soils sampled. Facultative aerobes and anaerobes are also present. (Mars, with its lack of oxygen, probably would support this latter group of organisms). By leaving the baffle pin in place (which was done in this test) a lower oxygen level prevailed, favoring the growth of an anaerobic population. The response obtained was less rapid and lower than usual. The antimetabolite continued to be effective. Upon completion of the test, segments of the soil-inoculated collecting line were removed aseptically from the incubating chamber and placed in an anaerobic liquid culturing medium (Difco Cooked Meat Medium) and incubated at room temperature. The resultant growth was inoculated into chambers containing tagged M8 medium, incubated aerobically and anaerobically (Brewer Jar), and the  $C^{14}O_2$  monitored. Ninety five cpm were detected from the aerobic growth, 1,155 cpm from the anaerobic, verifying the presence and predominance of anaerobic microorganisms in the field test culture chamber. In the field test, then, time had been required to deplete the oxygen already present in the chamber before the suitable environment for anaerobic metabolism and growth could be obtained.

The results of the field tests are presented in Figures 2-6. Responses were corrected for detection dead time.

FIGURE 2

FIELD TEST - GULLIVER III

Date: June 4, 1963

Weather: 22°C, sunny, slight wind

Location: Field - AMF

Orientation: Detector up

Ground Condition: Moist, packed soil

Soil Sample Collected: Fair

Medium: 3 ml, M8, 6.16 uc/ml

Antimetabolite: None used

Radioactive Substrates:

- (a) Formate - 0.13 mM, Sp. Act. 25.0 mc/mM, 3.30 uc/ml
- (b) Glucose - 0.14 mM, Sp. Act. 4.7 mc/mM, 0.66 uc/ml
- (c) Lactate - 0.44 mM, Sp. Act. 5.0 mc/mM, 2.20 uc/ml

Mechanical Function:

Components:

- (a) Sequencing: No problems arose
- (b) Projectiles: One projectile remained attached, released at port
- (c) Thermostat: Functioning
- (a) Detector: Geiger Müller tube
- (b) Collector: Gum coated, Ba(OH)<sub>2</sub>

General Evaluation: Antimetabolite was not used. One instrument inoculated, one used as sterile control. Sterile control level was satisfactory. Inoculated, uninhibited response was good.

Response:

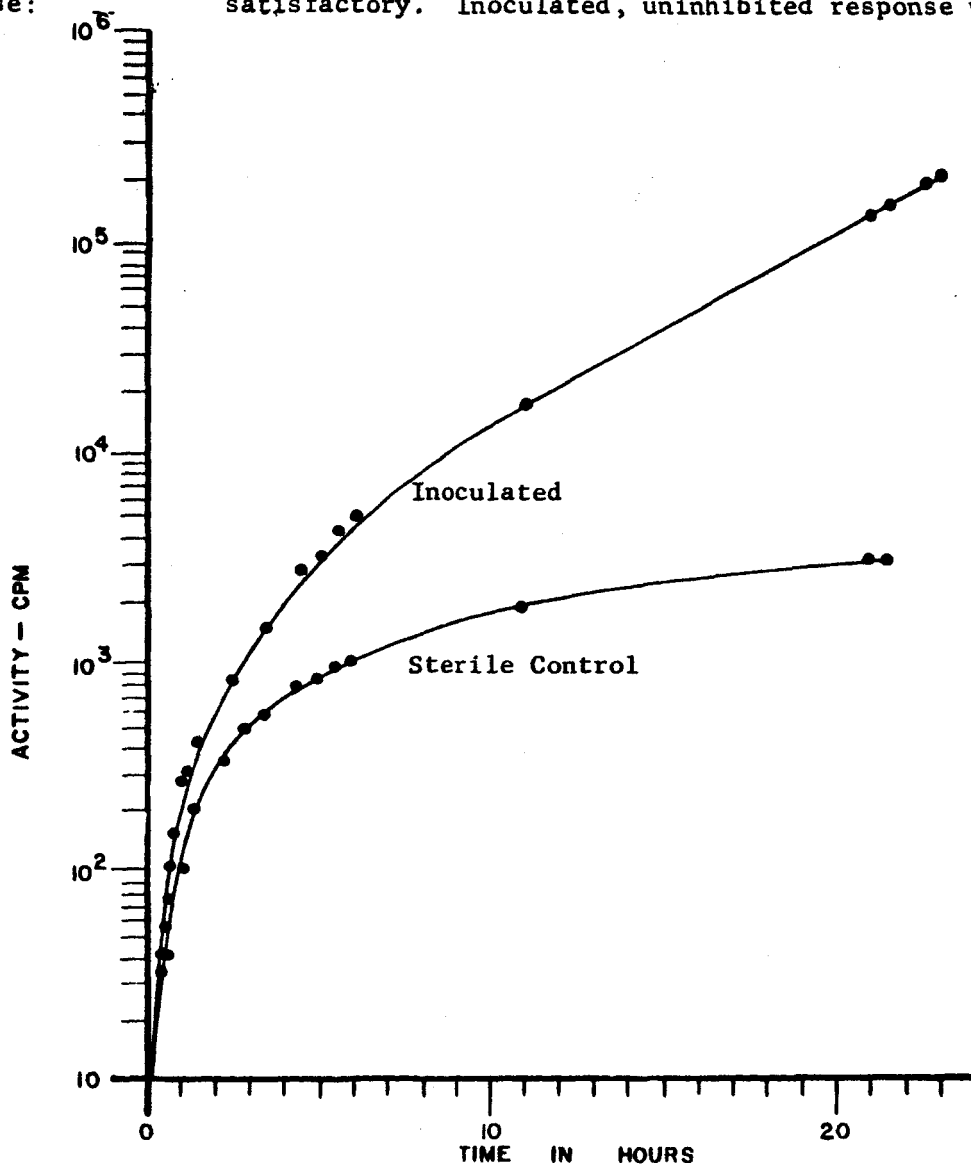


FIGURE 3

FIELD TEST - GULLIVER III

Date: June 19, 1963

Weather: 29°C, sunny, slight wind

Location: Upshur Recreation Area

Orientation: Detector up

Ground Condition: Dry, dusty

Soil Sample Collected: Very good

Medium: 3 ml, M8, 6.16 uc/ml

Antimetabolite: 0.5 ml, Bard Parker

Radioactive Substrates:

- (a) Formate - 0.13 mM, Sp. Act. 25.0 mc/mM, 3.30 uc/ml
- (b) Glucose - 0.06 mM, Sp. Act. 12.0 mc/mM, 0.66 uc/ml
- (c) Lactate - 0.44 mM, Sp. Act. 5.0 mc/mM, 2.20 uc/ml

Mechanical Function:

Components:

- (a) Sequencing: No problems arose
- (b) Projectiles: No problems arose
- (c) Thermostat: Functioning

- (a) Detector: Geiger Müller tube
- (b) Collector: Krylon, Ba(OH)<sub>2</sub>

General Evaluation: Mechanical operation was satisfactory. Low response due to predominance of anaerobic microorganisms and saturated collector. The antimetabolite was effective.

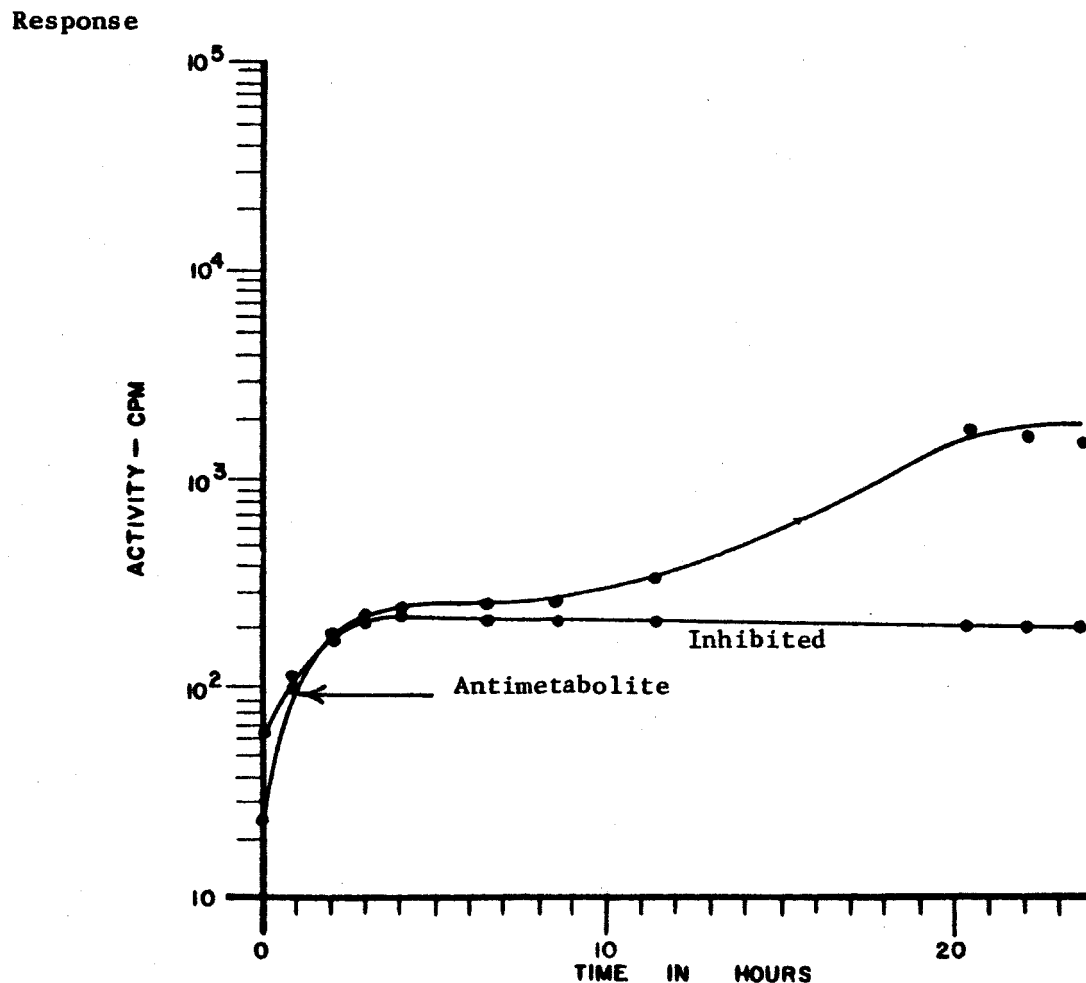


FIGURE 4

FIELD TEST - GULLIVER III

Date: June 27, 1963

Weather: 30°C, hot, sunny, no wind

Location: Upshur Recreation Area

Orientation: Detector up

Ground Condition: Dry, dusty

Soil Sample Collected: Very good

Medium: 3 ml, M8, 6.16 uc/ml

Antimetabolite: 0.5 ml Bard Parker

Radioactive Substrates:

- (a) Formate - 0.13 mM, Sp. Act. 25.0 mc/mM, 3.30 uc/ml
- (b) Glucose - 0.14 mM, Sp. Act. 4.7 mc/mM, 0.66 uc/ml
- (c) Lactate - 0.44 mM, Sp. Act. 5.0 mc/mM, 2.20 uc/ml

Mechanical Function:

- (a) Sequencing: No problems arose
- (b) Projectiles: No problems arose
- (c) Thermostat: Functioning
- (a) Detector: Geiger Müller tube
- (b) Collector: Krylon, Ba(OH)<sub>2</sub>

General Evaluation: Satisfactory, although collectors were saturated.

Response:

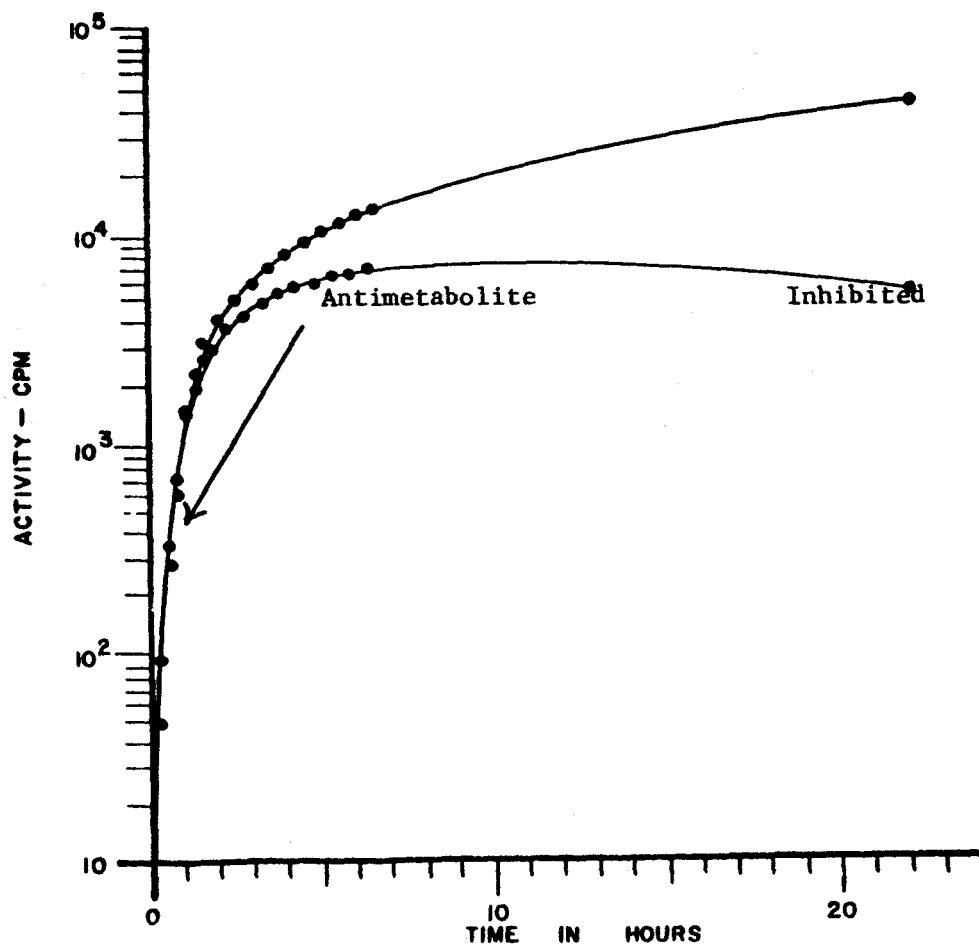


FIGURE 5  
FIELD TEST - GULLIVER III

Date: July 2, 1963

Weather: 33°C, sunny, slight wind

Location: Field - AMF

Orientation: Detector up

Ground Condition: Dry, dusty

Soil Sample Collected: Very good

Medium: 3 ml, M8, 6.16 uc/ml

Antimetabolite: 0.5 ml, Bard Parker  
injected at time zero

Radioactive Substrates:

- (a) Formate - 0.13 mM, Sp. Act. 25.0 mc/mM, 3.30 uc/ml
- (b) Glucose - 0.14 mM, Sp. Act. 4.7 mc/mM, 0.66 uc/ml
- (c) Lactate - 0.44 mM, Sp. Act. 5.0 mc/mM, 2.20 uc/ml

Mechanical Function:

Components:

- |                                    |  |
|------------------------------------|--|
| (a) Sequencing: No problems arose  | (a) Detector: Geiger Muller tube           |
| (b) Projectiles: No problems arose | (b) Collector: Krylon, Ba(OH) <sub>2</sub> |
| (c) Thermostat: Not connected      |  |

General Evaluation: Satisfactory, although collectors were saturated.

Response:

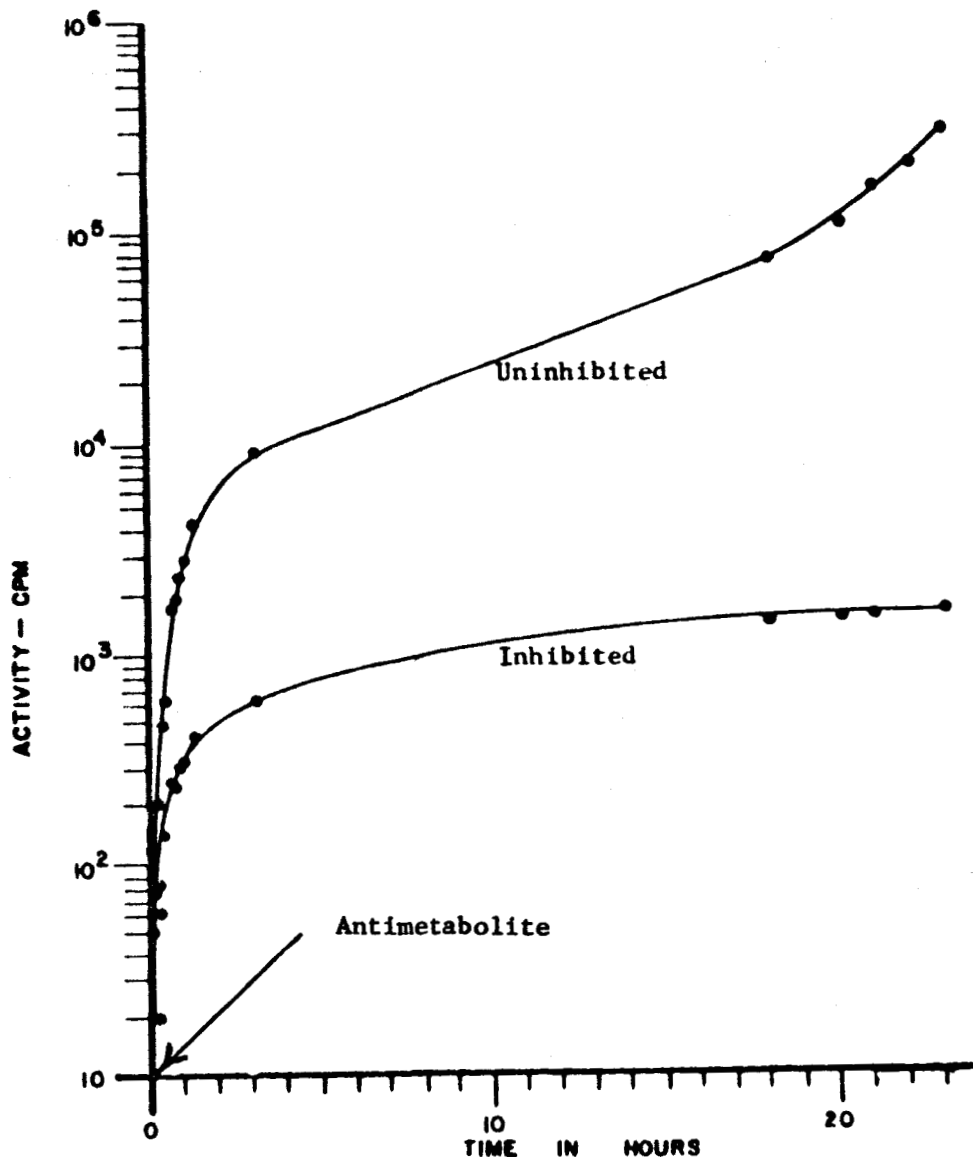


FIGURE 6  
FIELD TEST - GULLIVER III

Date: July 17, 1963

Weather: 33°C, sunny, slight wind

Location: Field - AMF

Orientation: Detector up

Ground Condition: Hard, dry, dusty

Soil Sample Collected: Very good

Medium: 3 ml, M8, 6.16 uc/ml

Antimetabolite: 0.5 ml, Bard Parker

Radioactive Substrates:

- (a) Formate - 0.13 mM, Sp. Act. 25.0 mc/mM 3.30 uc/ml
- (b) Glucose - 0.14 mM, Sp. Act. 4.0 mc/mM 0.66 uc/ml
- (c) Lactate - 0.44 mM, Sp. Act. 5.0 mc/mM 2.20 uc/ml

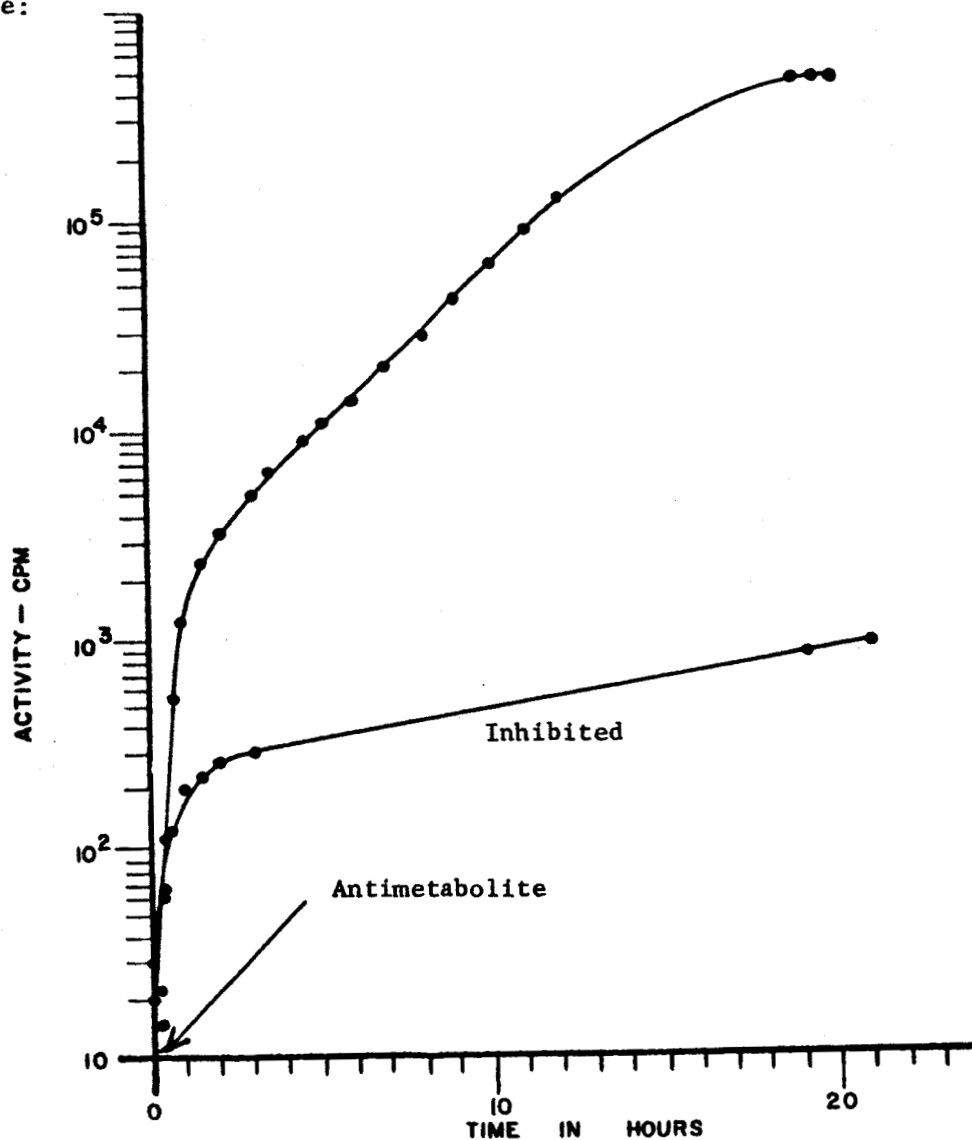
Mechanical Function:

Components:

- (a) Sequencing: No problems arose
- (b) Projectiles: One projectile remained attached, released at port
- (c) Thermostat: Not connected
- (a) Detector: Geiger Müller tube
- (b) Collector: AMF Tissuglas Ba(OH)<sub>2</sub>

General Evaluation: Satisfactory, although collectors became saturated.

Response:



A site has been selected for the first 'special environment' field test. According to the photometric and polarimetric measurements of several observers, the soil of Mars resembles that of pulverized limenine, an ore of ferric oxide. Limenine-rich soil was located, with the assistance of Maryland and Virginia state soil scientists, in Orange County, Virginia. The test is tentatively scheduled for the last week of September.

The shape of the curve obtained when responses are measured cumulatively in the automatic recording system and the Gulliver field test units differs in appearance from the shape of the curve obtained when incremental responses are plotted. Although it is obvious that the cumulative curve, when broken down and plotted incrementally, does indeed resemble the normal growth curve, no presentation of field test data in this manner has been made in the past. As a matter of interest, one field test is presented in Figure 6 in the cumulative manner and, in Figure 7 in an incremental manner. The resemblance to a standard growth curve is clear in Figure 7.

G. PERSONNEL, CONFERENCES, PUBLICATIONS

A. PERSONNEL

Duane G. Hoffman has become associated with the Radioisotopic Biochemical Probe for Extraterrestrial Life as Design Engineer. Mr. Hoffman is experienced in quality control procedures and will be responsible for these aspects of the program.

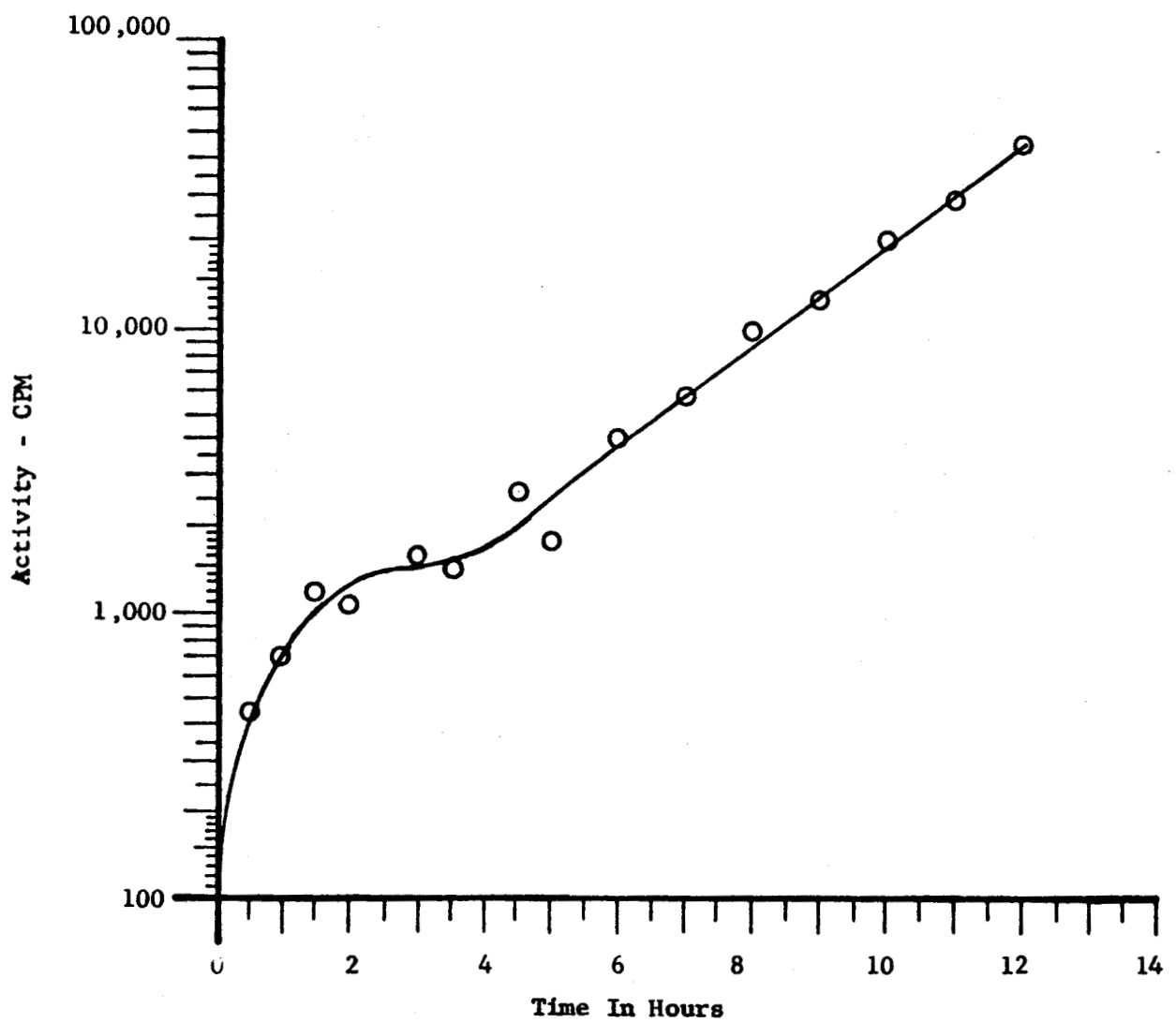
B. CONFERENCES

On July 10-12, 1963, conferences were held with personnel from Resources Research, Incorporated, American Machine and Foundry Company, Jet Propulsion Laboratory, and Dr. Norman Horowitz, California Institute of Technology, at the Jet Propulsion Laboratory, Pasadena, California.

Dr. L. Hochstein and Mr. J. Cole of the Ames Research Center, Palo Alto, California appraised the Gulliver experiment as part of an overall NASA review of space probe experiments on June 26, 27, 1963 at Resources Research, Incorporated. A field demonstration

FIGURE 7

FIELD TEST - JULY 17, 1963 - PLOTTED INCREMENTALLY



of the instrument was included. Personnel from Resources Research, Incorporated, American Machine and Foundry Company and Ames Research Center were in attendance.

C. PUBLICATIONS

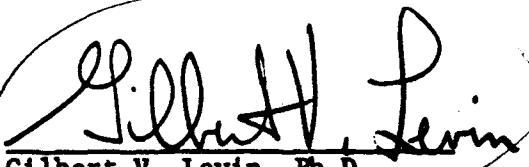
"Gulliver", An Experiment for Extraterrestrial Life Detection and Analysis was presented June 10, 1963, at the COSPAR Fourth International Space Science Symposium, Warsaw, Poland.

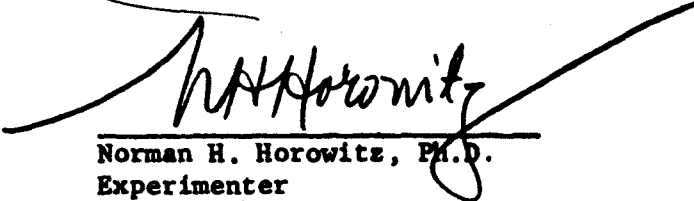
Quarterly Progress Report No. 10

NASr-10

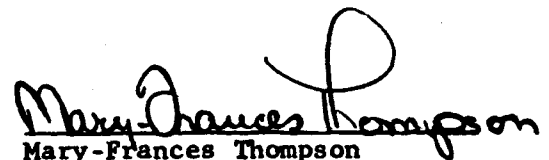
September 25, 1963

Respectfully submitted,

  
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PART III

INSTRUMENTATION



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### III. INSTRUMENTATION

#### A. GENERAL

During this reporting period there has been a re-direction of efforts to put more emphasis on the laboratory investigations of radioisotope detection, gas collection, nonmetabolic gas removal, and soil sample collection. To facilitate this effort, there has been a reduction of the local field testing activity.

The field tests that were performed early in the quarter entailed no mechanical or electronic difficulties. This situation indicates that a reasonable degree of reliability has been attained for purposes of evaluating minor changes in instrument components under terrestrial field test conditions.

The greatest emphasis of the laboratory efforts will be placed on improving radioisotope detection because preliminary tests indicated that sensitivity can be increased very significantly with a detector which permits using a larger gas collector and which has less window absorption than the end window geiger tube presently used. There have been several significant findings in the detector, gas collector, and nonmetabolic gas removal investigations which are discussed in the following sections. Optimization of the gas collection and nonmetabolic gas removal functions are extremely important facets of the task of improving Gulliver performance.

Plans are being made and equipment is being assembled and modified to operate two regular Gulliver III instruments and one uninoculated Gulliver III mock-up sterile control during each of the local and out-of-town field tests of the last half of the contract period. A low power, portable, automatic recording system is being assembled for sequential recording of the output of the three units used in the field test. This equipment will facilitate continuity in the acquisition of field test data without attendant personnel.

#### B. DETECTOR TESTS

The primary effort in the field of detector work in this quarter was to perform tests that would give an indication of the gain in sensitivity to be obtained by use of a windowless (proportional) counter rather than the conventional thin, end-window GM tube. The GM tubes used in these tests had a window thickness of about  $1.4 \text{ mg cm}^{-2}$ .

The experimental apparatus used to perform these tests was described and diagrammed in the last quarterly report. With this apparatus, many tests have now been completed, the results of which are given below. There are several variables in any one test: (1) Type of  $\text{C}^{14}\text{O}_2$  generation, i. e., metabolic or chemical; (2) amount of  $\text{C}^{14}\text{O}_2$  released; (3) amount and type of gas collector employed on the detectors; (4) the uniformity and reproducibility of these collectors. Item (4) appears to be a major hindrance in obtaining closely reproducible results in an investigation of this type.

The data are displayed in the following way: A summary of detector comparison tests is shown in Table III-1 and curves of the more significant tests are shown individually in Figures III-1 through III-10.

The dotted lines shown on the P.C. (windowless proportional counter) curves are a correction factor to the P.C. count rate. Because the system operates with no gas flow during the test, any leakage of air into the chamber degrades the P.C. performance. The amount of degradation can be determined by placing a small gamma source on the P.C. at the beginning and at various times during the test. The percent change from the initial rate observed in this gamma count is then the correction factor for the counter.

In Test 1, the collectors were made with the old technique of spraying krylon and  $\text{Ba}(\text{OH})_2$ . This test has limited significance because of the uncertain reliability of the spraying technique.

Tests 2 and 3 were performed to show the advantage of an internal gas counter if no collectors are used. The baffles were located so that approximately equal gas volumes were below each counter.

Tests 4 through 12 were performed with the recently developed hydroxide on Tissuglas pads. One pad was always placed on the GM window while the number of pads in P.C. was progressively increased to five, which covered most of the inner surface area of the counter.

There are three factors which enable the P.C. to be more sensitive than the GM under the present test conditions: (1) The lack of absorption in a window; (2) the ability to count a greater volume of  $\text{C}^{14}$  tagged gas in

**TABLE III-1**  
**SUMMARY OF DETECTOR COMPARISON TESTS**

Test No.	Collector	C <sup>14</sup> O <sub>2</sub> Source	No. Pads		Weight		Plateau cpm		Flushed cpm		See Figure No.	
			Tissuglas	Ba(OH) <sub>2</sub> mg	P.C.	G-M	P.C.	G-M	P.C.	G-M		
1	{ 30 mg Ba(OH) <sub>2</sub> and krylon on each detector	0.3 cc Na <sub>2</sub> C <sup>14</sup> O <sub>3</sub> (10 µc/cc)	1	1	35	35	128K	25K	72K	6.2K	35K	5.5K
2	{ No collectors	E. coli	1	1	36	36	5:1	5:1	12:1	12:1	6.4:1	
3	{ No baffles						68K	17K	28K	1.5K		
	{ No collectors	E. coli					4:1		19:1			
	{ With baffles						120K	5.5K	22K	0.5K		
							22:1		44:1			
							13:1					
4	1	1	35	35	0.2 cc Na <sub>2</sub> C <sup>14</sup> O <sub>3</sub>	128K	25K	58K	20K			III-1
5	1	1	36	36	"	5:1	5:1	3:1 per mg	3:1 per mg			III-2
6	2	1	72	36	"	4:1	4:1	2.3:1 per mg	2.3:1 per mg			III-3
7	2	1	58	27	"	22:1	22:1	7:1 per mg	7:1 per mg			III-4
8	3	1	120	50	"	13:1	13:1	3:1 per mg	3:1 per mg			III-5
9	3	1	93	33	"	13:1	13:1					III-6
10	5	1	201	47	"	19:1	19:1	44K	4.4K			III-7
11	5	1	80	13	"	116K	4.3K	3.4:1 per mg	3.4:1 per mg			III-8
12	5	1	78	19	"	27:1	27:1	80K	4.1K			III-9
13	3	1	77	26	E. coli	4.6:1 per mg	4.6:1 per mg	4.6:1 per mg	4.6:1 per mg			III-10
						105K	105K	3.5:1 per mg	3.5:1 per mg			
						33K	33K	3.7K	3.7K			
						2.25:1 per mg	2.25:1 per mg					
						30K	30K					
						10:1	10:1					

	<u>P.C.</u>	<u>G-M</u>
Number of Pads:	1	1
mg of Ba(OH) <sub>2</sub> :	35	35
C <sup>14</sup> O <sub>2</sub> Source:	3μc C <sup>14</sup> in 3 m mole Na <sub>2</sub> C <sup>14</sup> O <sub>3</sub> onto citric acid crystals.	

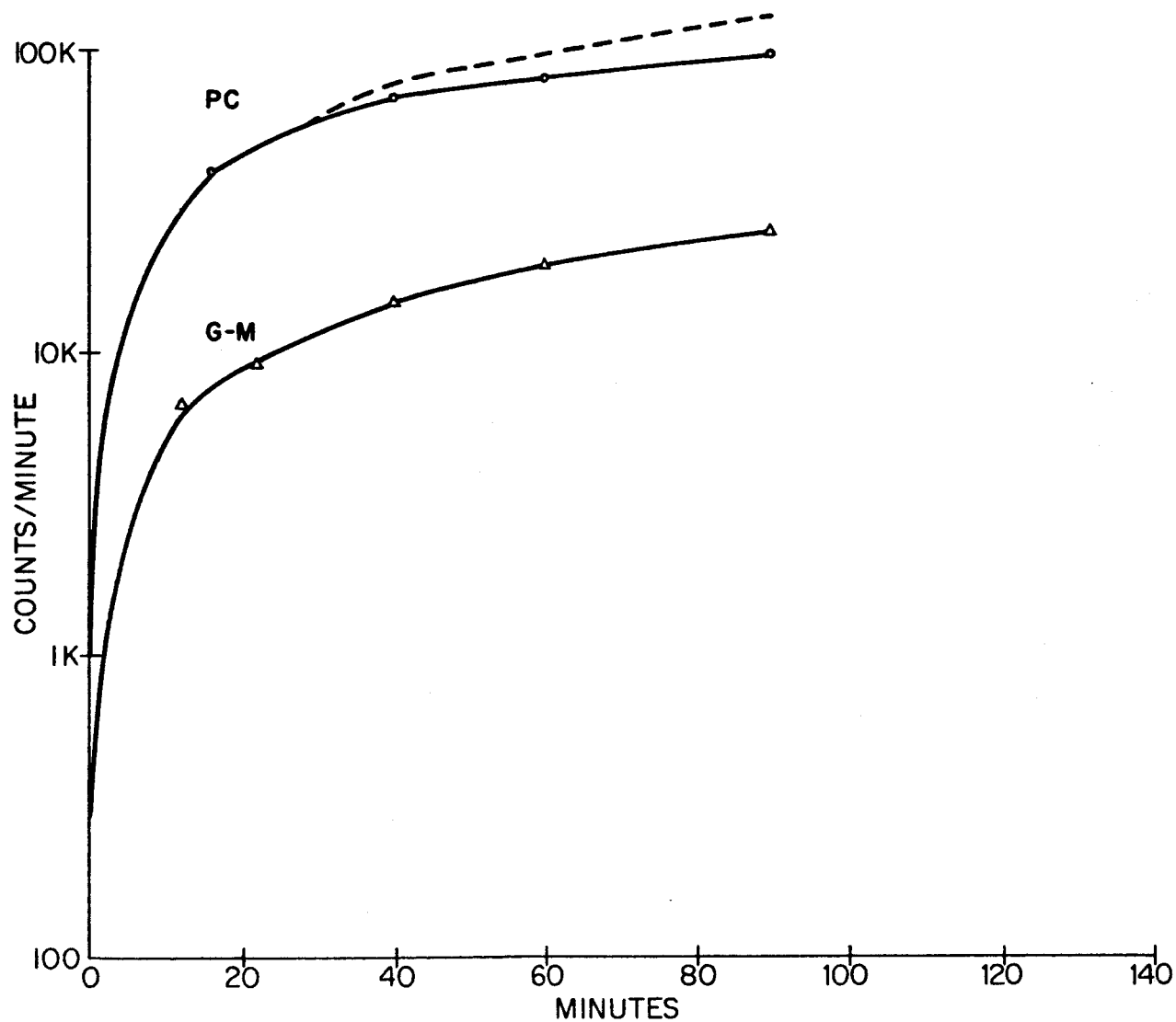


Figure III-1. Response of internal flow detector (P.C.) vs. end window Geiger tube (G-M) with identical C<sup>14</sup>O<sub>2</sub> exposures.

Number of Pads:	<u>P.C.</u> 1	<u>G-M</u> 1
mg of Ba(OH) <sub>2</sub> :	36	36
C <sup>14</sup> O <sub>2</sub> Source:	Same as III-1.	

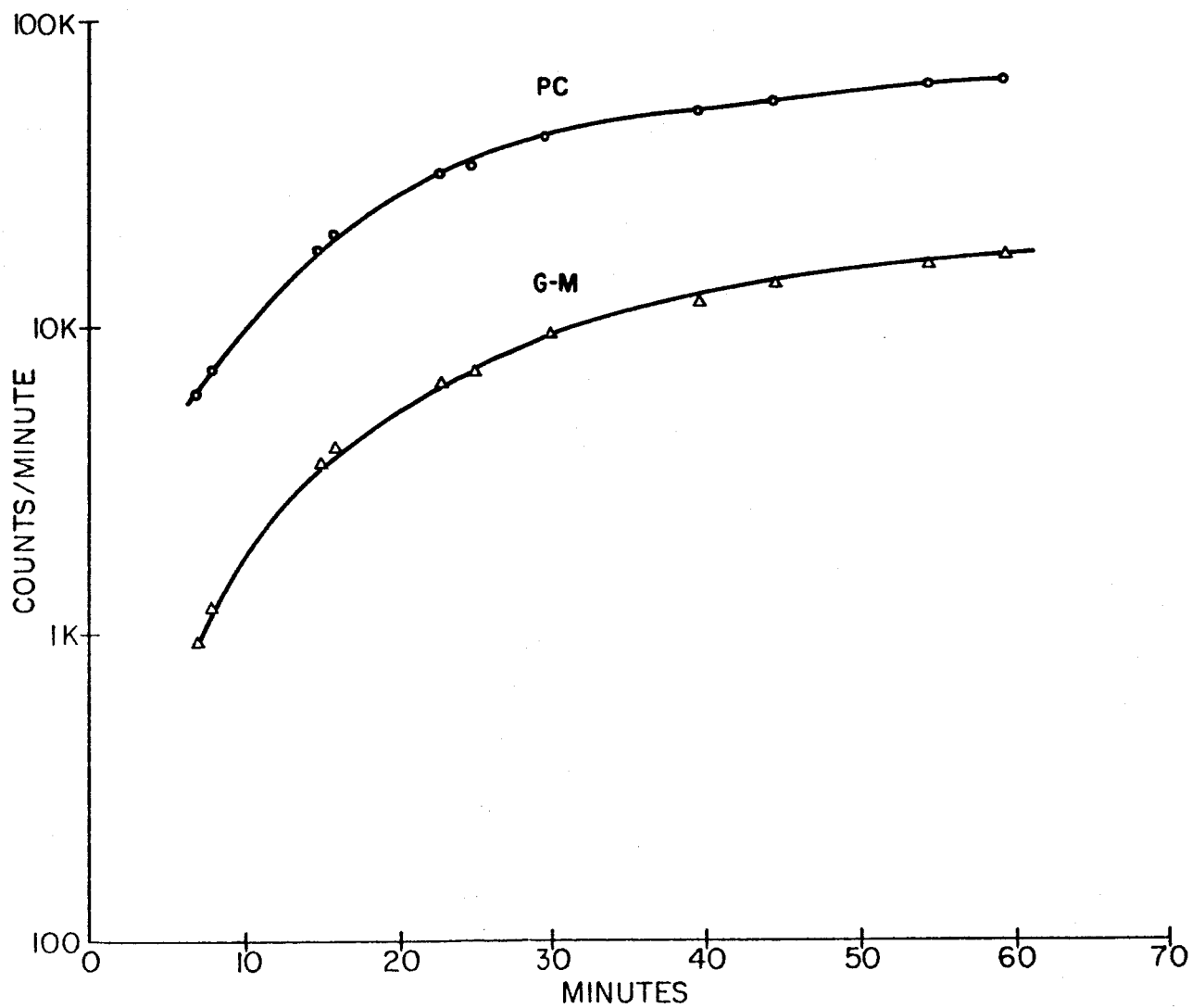


Figure III-2. Response of internal flow detector (P.C.) vs. end window Geiger tubes (G-M) with identical C<sup>14</sup>O<sub>2</sub> exposures.

	<u>P. C.</u>	<u>G-M</u>
Number of Pads:	1	1
mg of Ba(OH) <sub>2</sub> :	72	36
C <sup>14</sup> O <sub>2</sub> Source:	Same as III-1.	

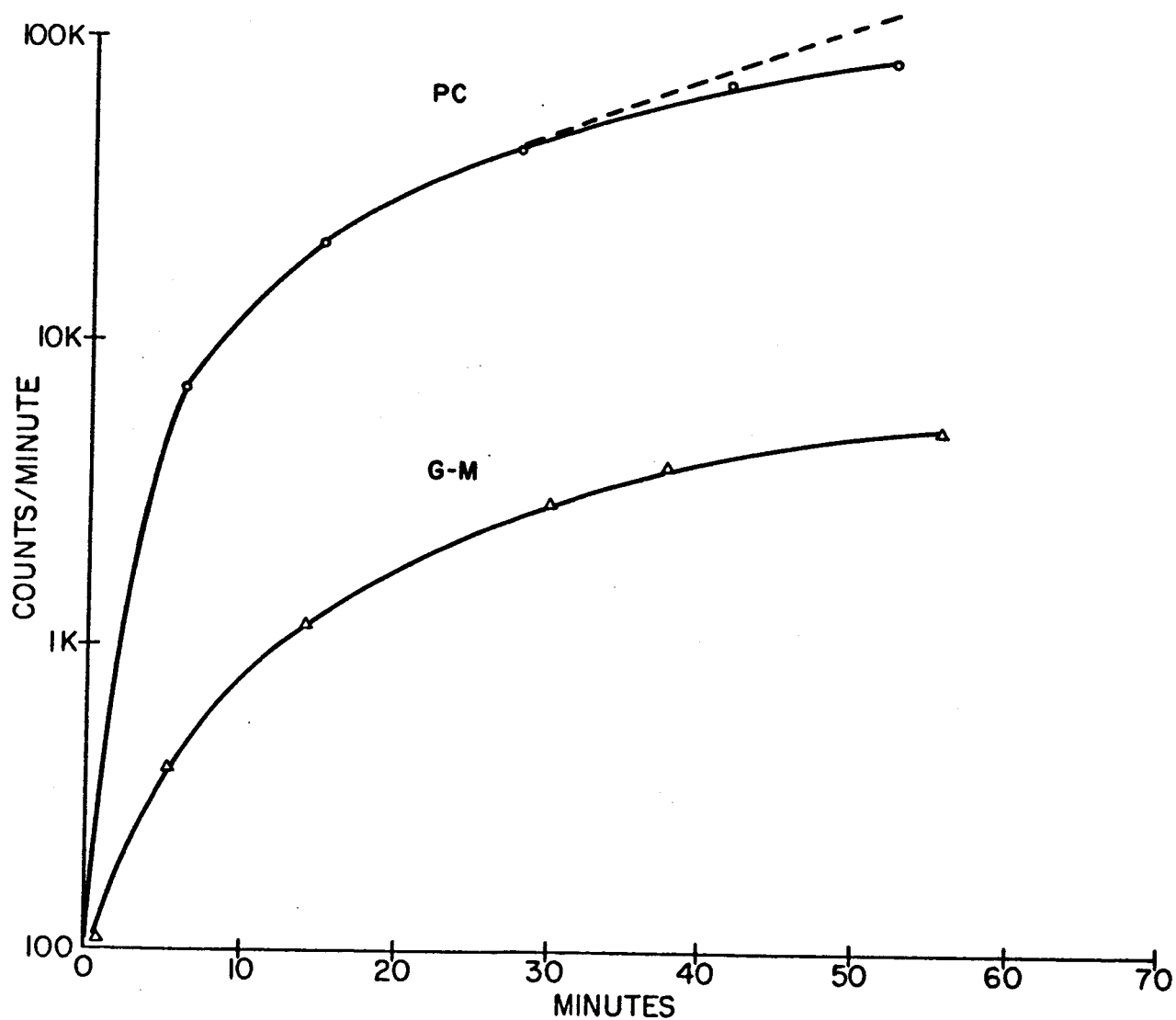


Figure III-3. Response of internal flow detector (P. C.) vs. end window Geiger tubes (G-M) with identical C<sup>14</sup>O<sub>2</sub> exposures.

Number of Pads:	<u>P.C.</u> 2	<u>G-M</u> 1
mg of Ba(OH) <sub>2</sub> :	58	27
C <sup>14</sup> O <sub>2</sub> Source:	Same as III-1.	

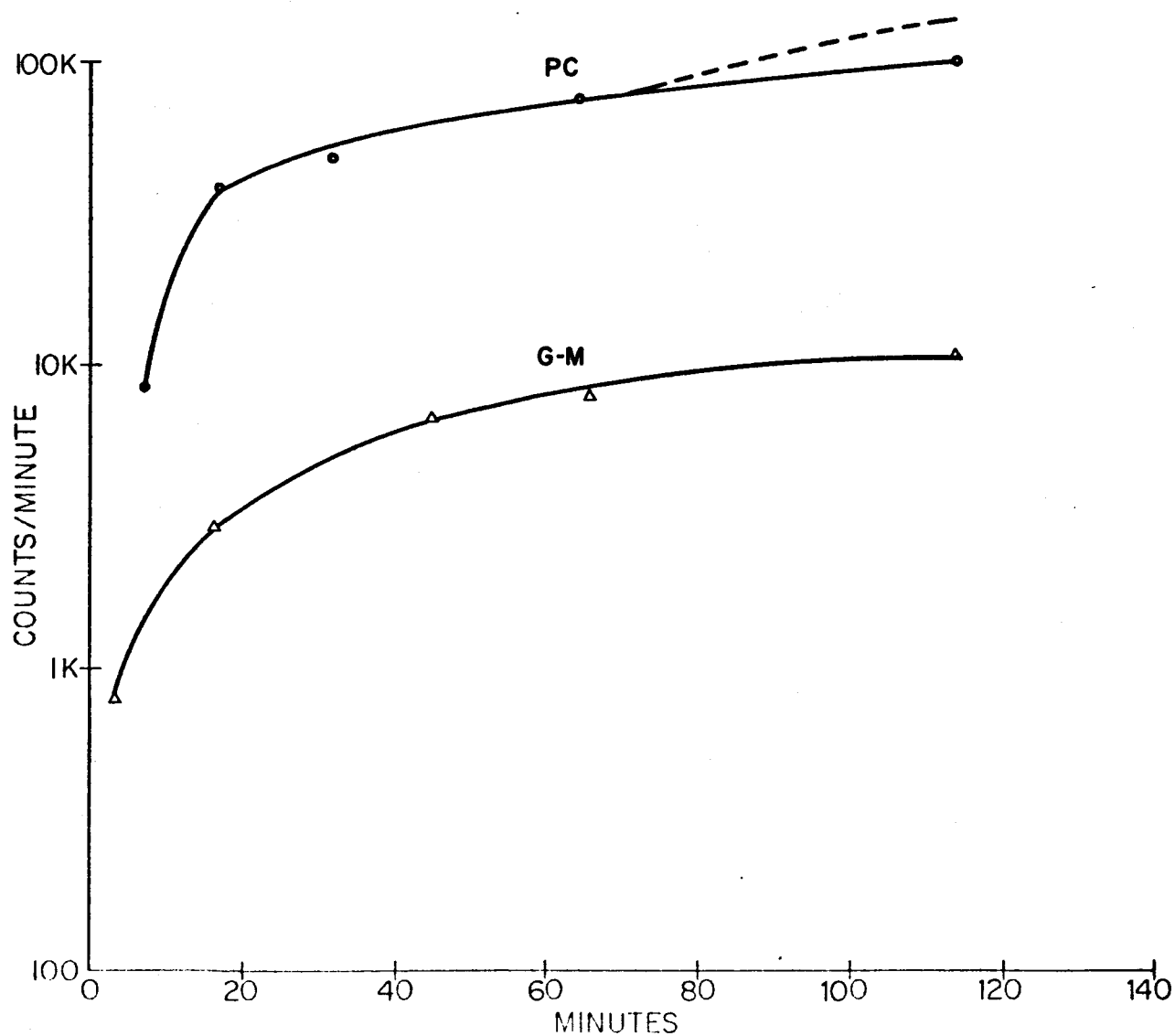


Figure III-4. Response of internal flow detector (P.C.) vs. end window Geiger tube (G-M) with identical C<sup>14</sup>O<sub>2</sub> exposures.

Number of Pads:	<u>P.C.</u> 3	<u>G-M</u> 1
mg of Ba(OH) <sub>2</sub> :	120	50
C <sup>14</sup> O <sub>2</sub> Source:	Same as III-1.	

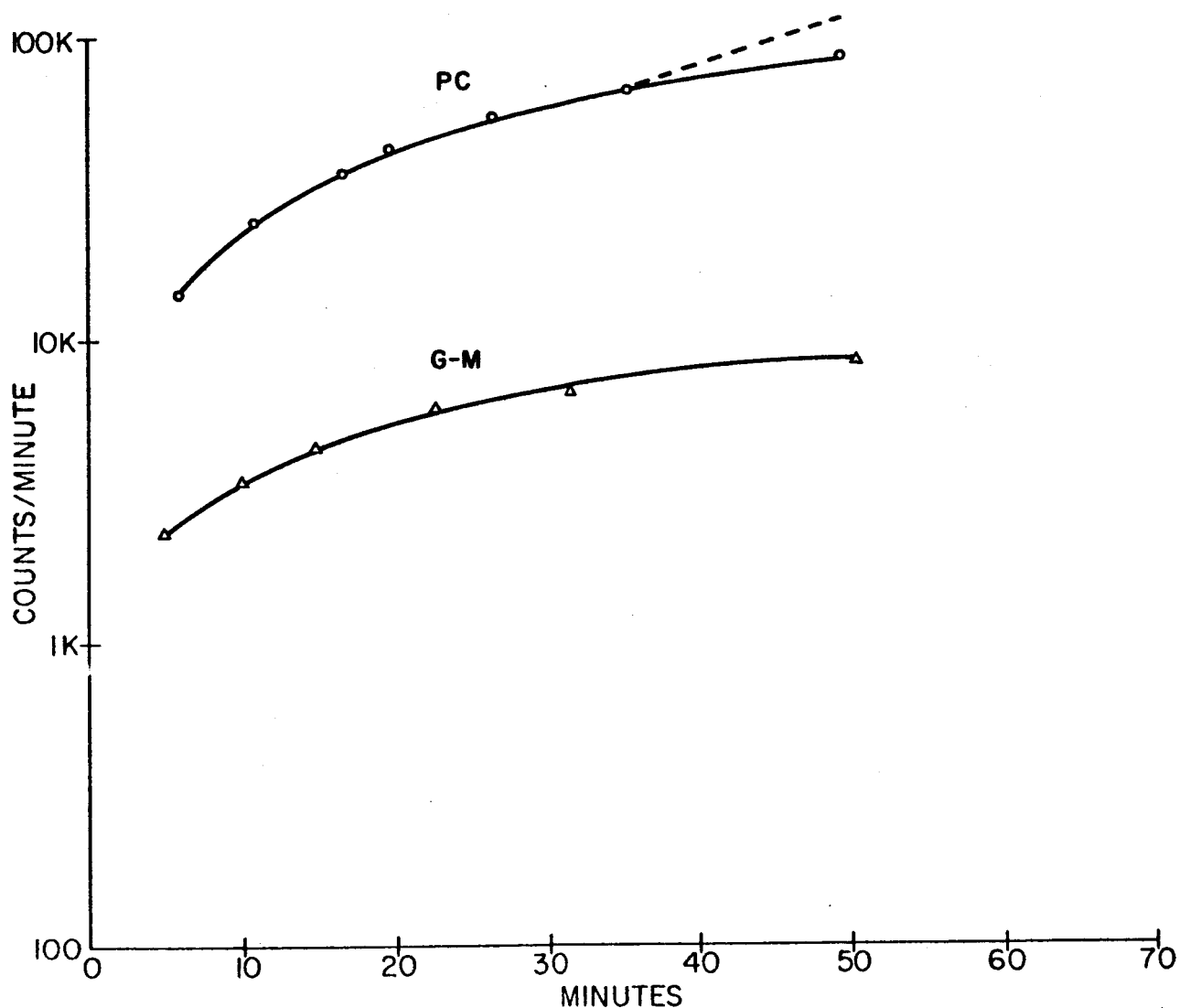


Figure III-5. Response of internal flow detector (P.C.) vs. end window Geiger tube (G-M) with identical C<sup>14</sup>O<sub>2</sub> exposures.

Number of Pads:	<u>P.C.</u> 3	<u>G-M</u> 1
mg of Ba(OH) <sub>2</sub> :	93	33
C <sup>14</sup> O <sub>2</sub> Source:	Same as III-1.	

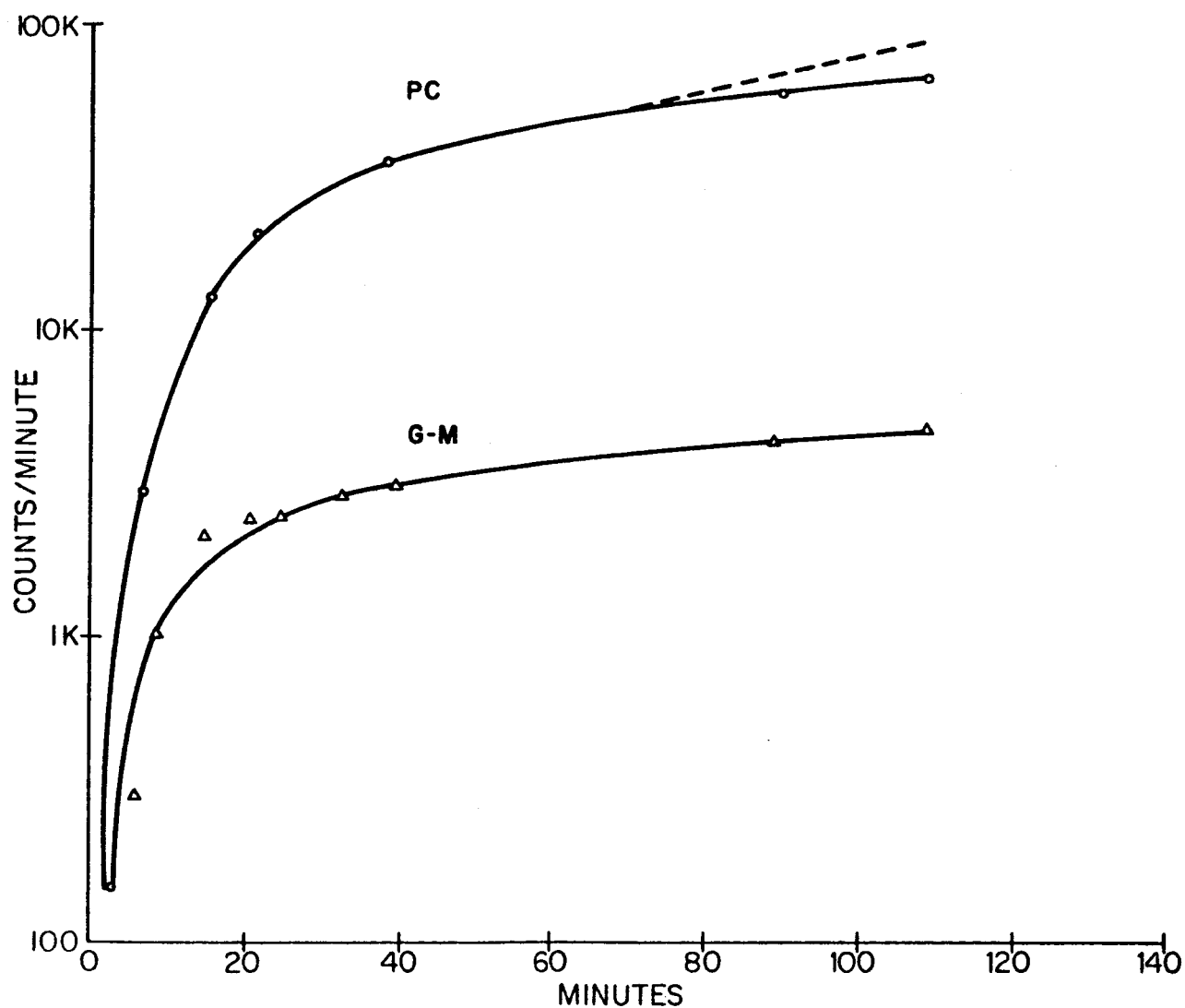


Figure III-6. Response of internal flow detector (P.C.) vs. end window Geiger tube (G-M) with identical C<sup>14</sup>O<sub>2</sub> exposures.

	<u>P. C.</u>	<u>G-M</u>
Number of Pads:	5	1
mg of Ba(OH) <sub>2</sub> :	201	47
C <sup>14</sup> O <sub>2</sub> Source:	Same as III-1.	

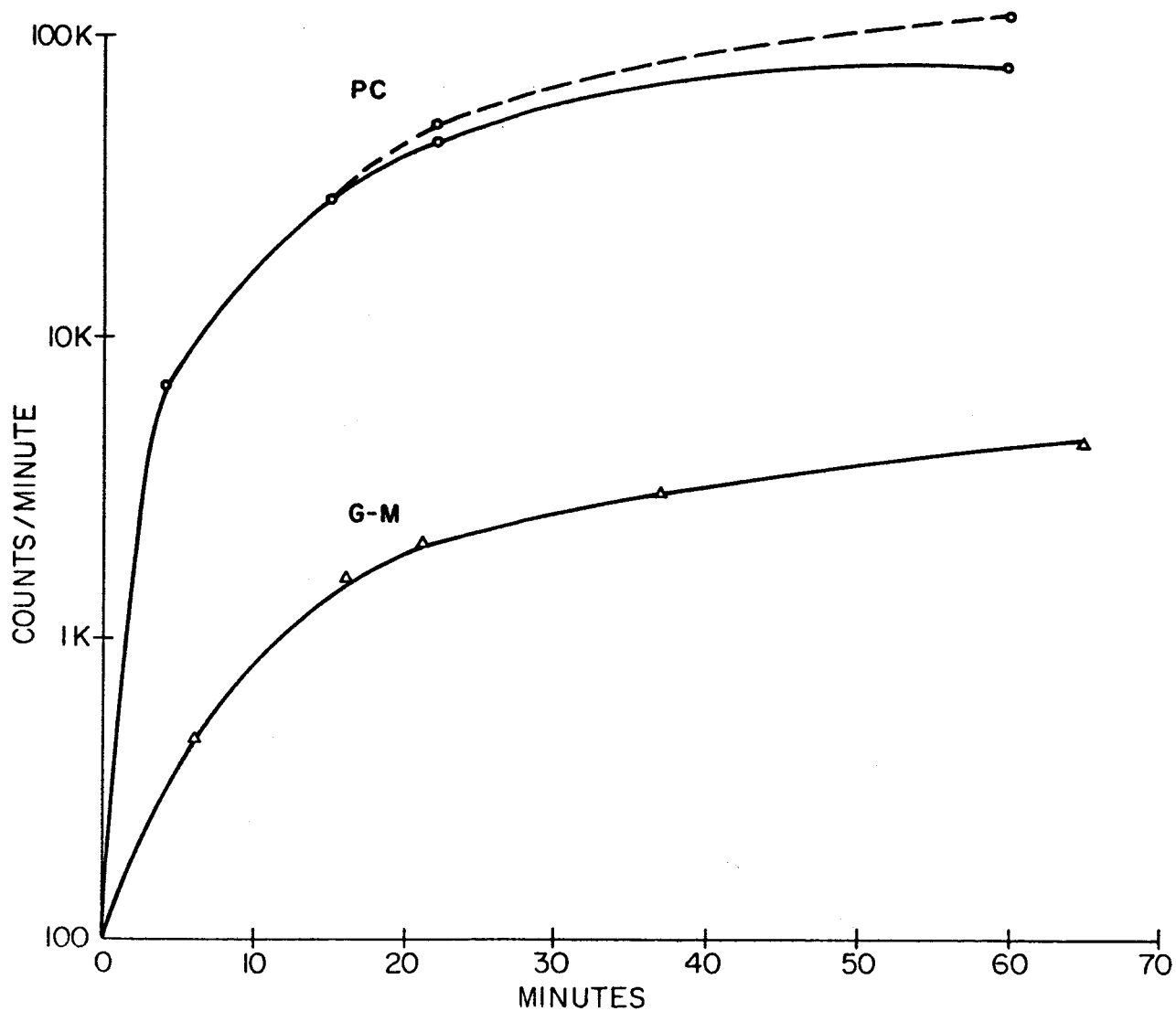


Figure III-7. Response of internal flow detector (P. C.) vs. end window Geiger tube (G-M) with identical C<sup>14</sup>O<sub>2</sub> exposures.

	<u>P.C.</u>	<u>G-M</u>
Number of Pads:	5	1
mg of Ba(OH) <sub>2</sub> :	80	13
C <sup>14</sup> O <sub>2</sub> Source:	Same as III-1.	

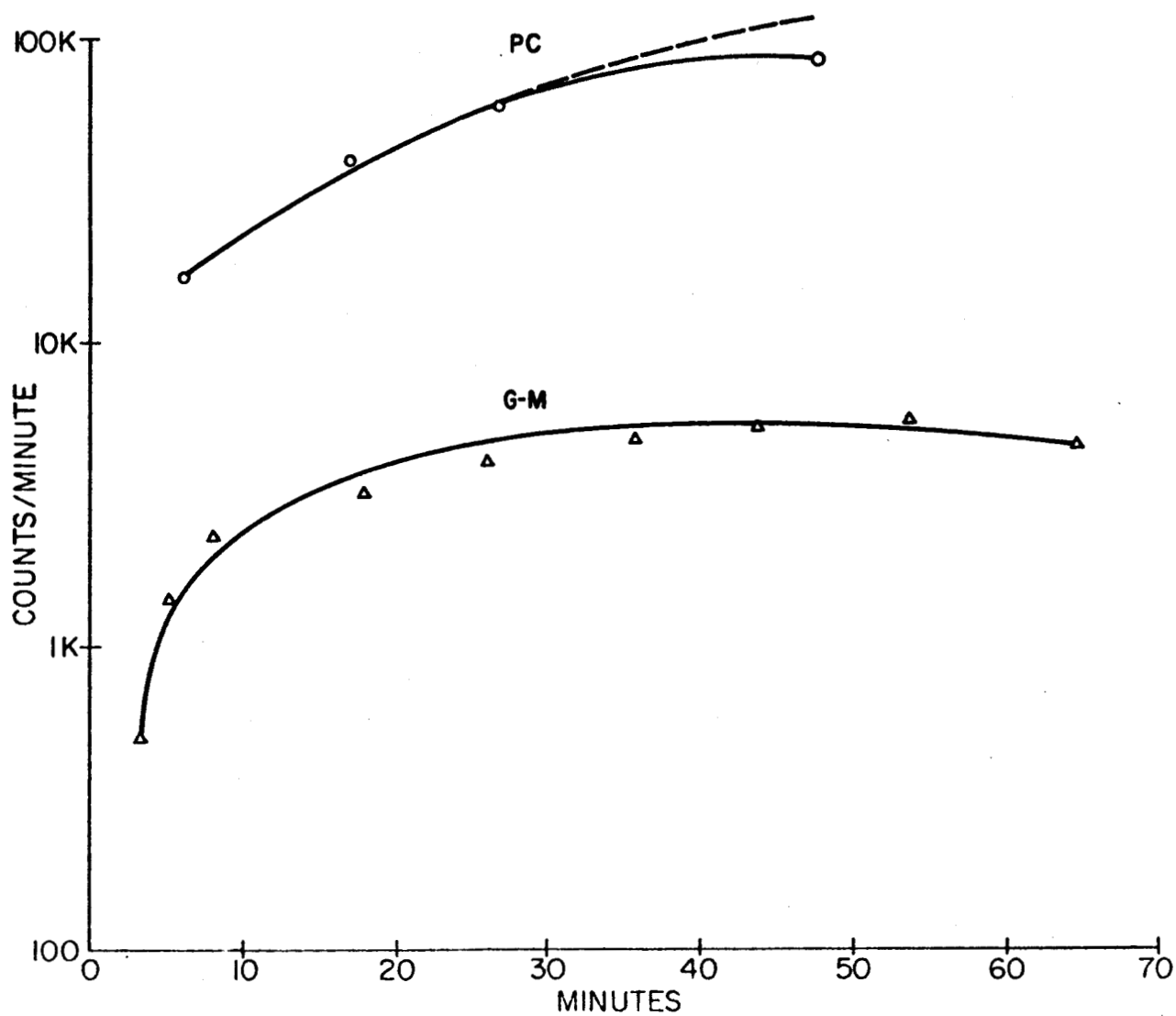


Figure III-8. Response of internal flow detector (P.C.) vs. end window Geiger tube (G-M) with identical C<sup>14</sup>O<sub>2</sub> exposures.

	<u>P.C.</u>	<u>G-M</u>
Number of Pads:	5	1
mg of Ba(OH) <sub>2</sub> :	78	19
C <sup>14</sup> O <sub>2</sub> Source:	Same as III-1.	

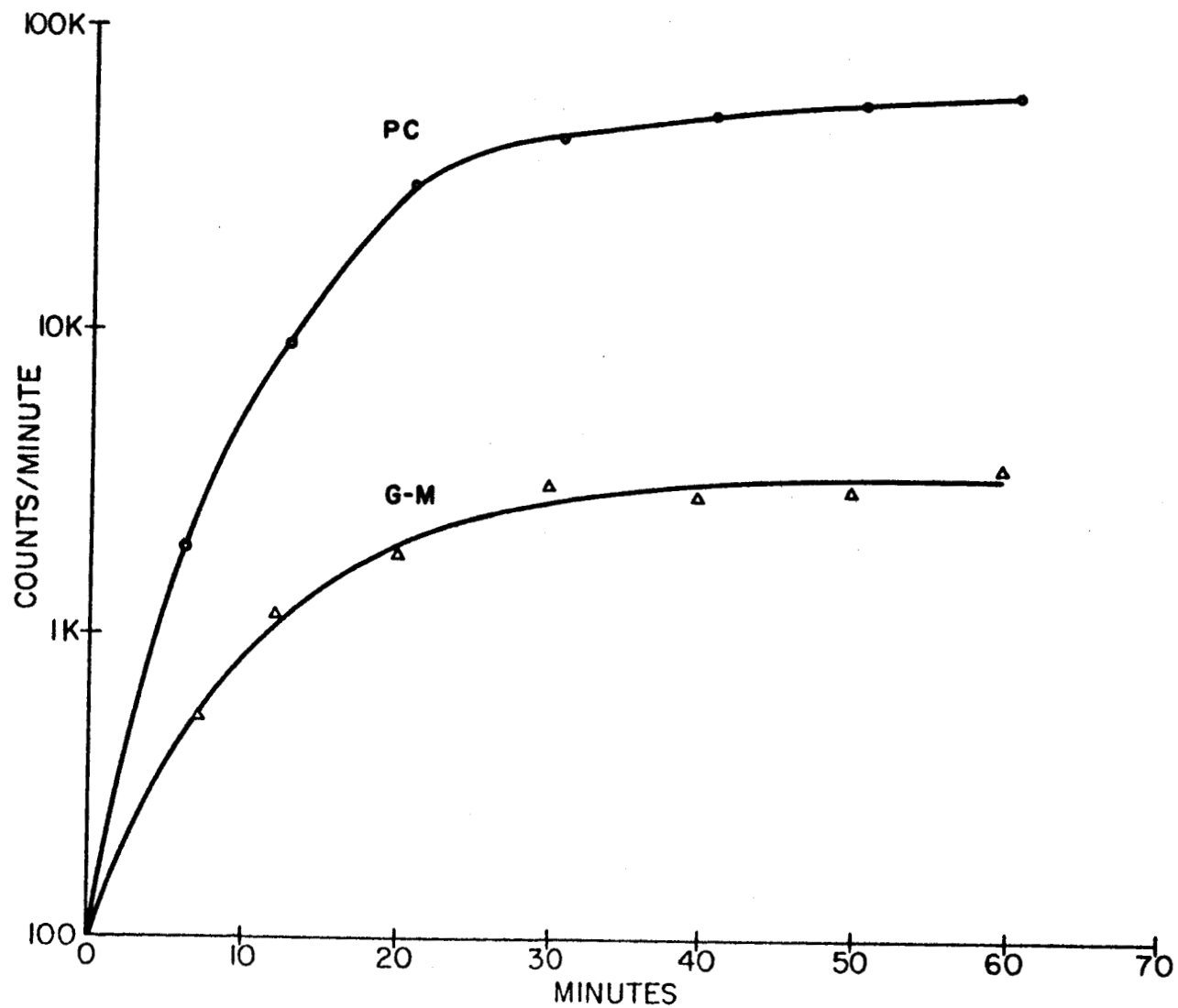


Figure III-9. Response of internal flow detector (P.C.) vs. end window Geiger tube (G-M) with identical C<sup>14</sup>O<sub>2</sub> exposures.

	<u>P.C.</u>	<u>G-M</u>
Number of Pads:	3	1
mg of Ba(OH) <sub>2</sub> :	77	26
C <sup>14</sup> O <sub>2</sub> Source:	E. coli + tagged medium.	

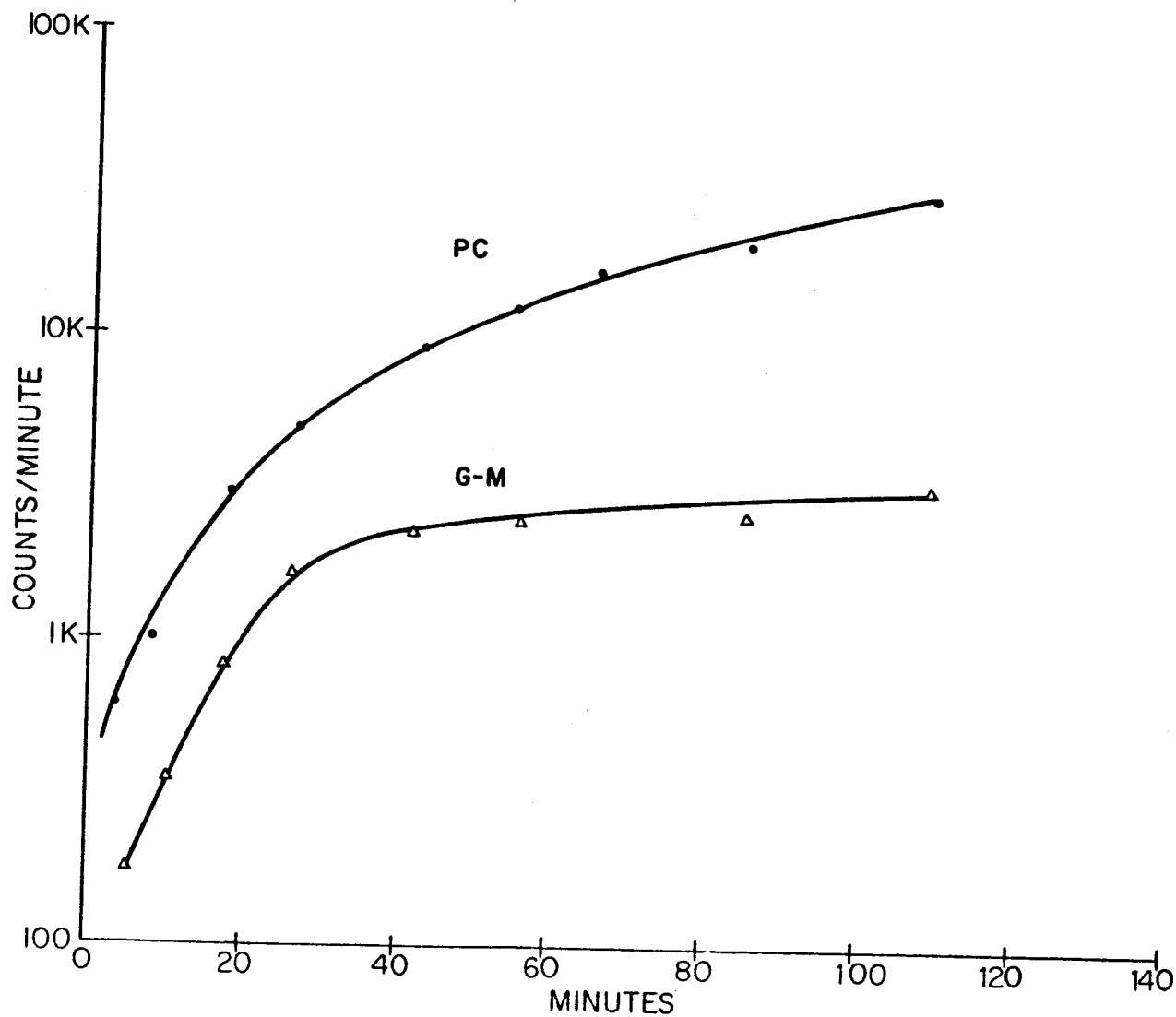


Figure III-10. Response of internal flow detector (P.C.) vs. end window Geiger tube (G-M) with identical C<sup>14</sup>O<sub>2</sub> exposures.

the gas phase with higher efficiency; and (3) the fact that the inner surface area is larger than the GM window area allows more gas collector to be utilized.

The "plateau" count rate refers to the rate obtained at some arbitrary time after both detectors had reached a point where their count rates were increasing relatively slowly.

The "flushed" count rate is that obtained with no gas phase counting. These count rates were obtained by removing all  $C^{14}O_2$  counting by flushing the P.C. with counting gas and removing the G-M from the test chamber with all pads kept in place. Thus, the only activity present--other than background and contamination--is that due to  $BaC^{14}O_3$  on the pads.

If the gross "flushed" count rate ratio is then divided by the ratio of the amount of  $Ba(OH)_3$  initially on the P.C. to that initially on the G-M, the flushed count rate per mg is obtained. On the basis of tests performed with three different internal flow counters and several  $BaC^{14}O_3$  samples, this ratio was found to be between 2:1 and 3:1, depending on the amount of self-absorption in the source.

The correlation between tests with similar ratios of hydroxide leaves much to be desired. The explanation for this spread is indicated by the "flushed" count rate ratio per milligram of collector. If the collectors were truly reproducible, this ratio should remain between 2:1 and 3:1. Since tests 7 and 10, for example, show very high plateau count ratios but

their per mg ratio is excessive, which indicates that the former ratio was high probably because of gas collector irregularities.

These data point up the necessity for more reproducible gas collectors since detector tests are obviously hampered with the present collectors. It is hazardous to attempt to draw firm conclusions from these comparisons because of these gas collector or other problems. These data do point, however, to a sensitivity gain of around 20:1 with a P.C. volume of 29 cc. Larger-volume counters should improve this further because of the additional gas phase counting and increased surface area for gas collector material.

Tests were also undertaken to determine if there would be any advantage in leaving a part of a window uncovered by a collector. The reason for performing these tests is that if the collector were to become saturated, then a partly uncovered window would be able to count the gas phase much more efficiently than a window entirely covered by the collector.

Three tests were performed to resolve this choice by generating more  $\text{CO}_2$  than the collectors could absorb on detectors partially and completely covered, but the resulting data were contradictory. It is felt that this was due to the fact that the "one shot" chemical evolution of  $\text{C}^{14}\text{O}_2$  which was utilized gave a response rate that was too rapid to observe. Tests will be accomplished in the near future with metabolic evolution or by a method of generating chemically in increments. These will also be with

excess  $\text{CO}_2$  so as to saturate the collectors. The slower  $\text{CO}_2$  generation should allow observation of the effect of the partly covered window.

### C. GAS COLLECTION

At the time of the last report, the gas collector work was being directed toward improvement of adhesive for the barium hydroxide in an attempt to get reproducible collectors. The experiments had shown an increased count rate by a factor of about five when the hydroxide was held by cellulose gum rather than krylon. At the beginning of this contract period, a different experiment was devised in which the two types of collector adhesives could be compared in a physical situation similar to the actual  $\text{CO}_2$  absorption in Gulliver III. An incubation chamber was made, as shown in Figure III-11, by bending a glass tube  $90^\circ$ , with about two inches of tubing for each side. A culture of bacteria plus tagged broth were placed on a piece of sterile sponge in the bend and two geiger tubes, one with cellulose gum and hydroxide collector and the other with krylon and hydroxide collectors, were sealed in the ends of the bent tube. Counts were made as a function of time as the culture grew and gave off tagged gases. Baffles at the faces of the geigers prevented detection of the betas from the broth itself.

Besides a reproducibility check for the bent-tube chamber itself, using identical collectors, three runs were made comparing krylon and cellulose gum adhesive collectors. In this metabolic generation of  $\text{C}^{14}\text{O}_2$ , the krylon/hydroxide collectors showed a definite superiority over the

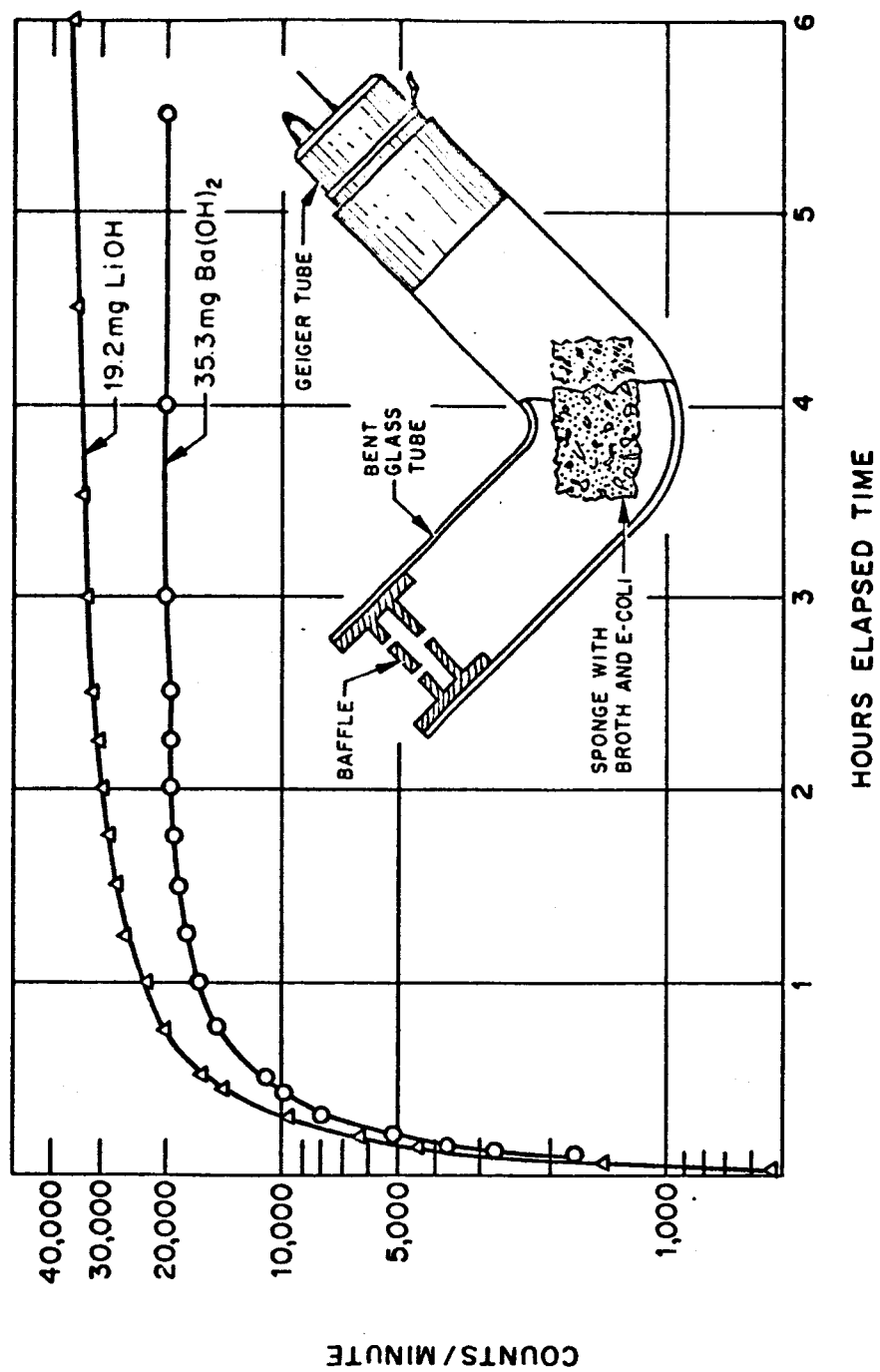


Figure III-11. Comparison of LiOH and Ba(OH)<sub>2</sub> collector pads on incubation chamber.

gum hydroxide in all three runs. This was surprising in light of previous experiments using chemically generated  $C^{14}O_2$ , exposing window planchets\* in a desiccator in which the cellulose gum and hydroxide mixture collectors appeared far better. Another run was made with the chemical generation method with the same, contradictory, result. A possible explanation is that the metabolic gases included substances which in some manner reacted with the gum/hydroxide coating to reduce its effectiveness but did not react as much with the hydroxide on the krylon surface. This discrepancy for chemical and metabolic generated  $CO_2$  requires further investigation. At any rate, since the incubation type of experiment is so closely related to the actual Gulliver III conditions, these data were considered superior to the chemical generation data. Consequently, the gum adhesive no longer seemed to hold promise. Although the cellulose gum adhesive was suitable for terrestrial field tests, it is not one of the JPL preferred adhesive materials for flight conditions. No further investigations were made with this substance.

To check for reproducibility and for optimum weight of krylon/hydroxide coatings, groups of window planchets were coated and exposed simultaneously to the same tagged  $CO_2$  atmosphere. They were then counted and exposed to further generation of  $CO_2$  and recounted. Plots of count rate

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\* A "window planchet" is a standard one-inch diameter aluminum planchet with a 3/4-inch hole cut in the bottom. This permits collectors to be exposed to tagged  $CO_2$  atmospheres in the test chamber and monitored from the unexposed side without removal from the chamber.

as a function of collector weight revealed that essentially equal weights could differ in count rate by a factor as high as 2.5, though more commonly 20 percent variation occurred. Conclusions from these experiments were that the krylon/barium hydroxide coating exhibits only fair reproducibility and that total  $\text{CO}_2$  absorption increases with increasing weight of collector up to the limit at which the coating no longer adheres to the mylar windows.

Several possible schemes for adhesion of collector were considered. One method would be to use a slower drying adhesive than krylon so that time would be available for a careful uniform-coating procedure. Along this line would be the use of a nondrying adhesive such as Minnesota Mining & Manufacturing Adhesive Transfer Tape. A second general approach would be to form the hydroxide into thin wafers by pressing, perhaps with a binding agent. A third method would be to suspend the hydroxide in a matrix of low density fibers. Because no work had been done even related to the latter two methods, these were looked into through a short experimental investigation.

A hydraulic press was used to press barium hydroxide powder into discs. Various attempts were made, including the use of binding agents. Though it appeared that thin discs of hydroxide could thus be formed of appropriate weight, they were very fragile and had hard, slick surfaces which looked nonporous. Because these discs appeared unsuitable, no gas absorption experiments were conducted in an attempt to devote the time to a more likely prospect: the suspension of hydroxide in a fiber matrix.

Noting that barium hydroxide is soluble in any desired proportion in water beyond 78°C, it seemed that by dipping fiberglass tissue paper in various solution strengths, weights of hydroxide absorbed by the tissue could be controlled as desired. This proved to be the case. Several types of tissue were tried and the one selected for initial absorption experiments on the basis of capacity, strength, and general appearance was an AMF product, "200 G Tissuglas".

The first quantitative experiment with Tissuglas was to check reproducibility. The Tissuglas was dipped in a  $\text{Ba}(\text{OH})_2$  solution, dried in a desiccator, and cut into round pads to fit over the holes in window planchets. Weight of hydroxide was determined by weighing each pad and subtracting the weight of a disc of Tissuglas alone. These planchets were exposed to the same chemical generation of tagged  $\text{CO}_2$  and the count rates compared. The results were encouraging from a reproducibility standpoint, with nearly equal weights exhibiting count rates within around 20 percent of each other.

Once reproducibility of barium hydroxide/Tissuglas pads was found reasonably satisfactory, experiments were carried out to compare the 200 G sensitivity to the previous "standard", krylon/hydroxide, and to another kind of Tissuglas, 60 G. At the same time, solution strength was also included as a parameter. Both metabolic and chemical generation of  $\text{CO}_2$  led to these conclusions: (1) the thinner tissue (60 G) is more sensitive,

but the thicker holds more hydroxide and has more absorptive capacity; (2) a solution strong enough to deposit around 25 mg of barium hydroxide on a 200 G pad is better than a weaker one; (3) in the most reliable test, using metabolic  $\text{CO}_2$ , the sensitivity of the barium hydroxide pad is similar to the sensitivity of krylon/barium hydroxide.

The field test on 17 July 1963 was the first time the pads were used in Gulliver III. The test was quite pleasing in that the live unit showed a fast response (500 cpm in 30 minutes; 1400 cpm in one hour; 3,000 cpm in four hours; 100,000 cpm in eleven hours) and a high absorptive capacity of nearly 500,000 cpm at saturation.

The collector pads have proven to have other advantages in addition to being relatively sensitive, having high absorptive capacity, and being fairly reproducible. The weight of tissue is a known quantity, so that the exact amount of hydroxide can be determined by weighing a pad. By making a quantity of pads, the desired weights can be selected from the distribution, including close, if not exact duplicates. The pads can be made in advance and used when desired if they are stored in an airtight container containing a  $\text{CO}_2$  absorber. The last-named advantage was proven by an experiment covering twelve days of storage.

The low melting point ( $78^\circ\text{C}$ ) of barium hydroxide and its high molecular weight led back to the consideration of lithium hydroxide as a collector. The sterilization temperature of  $135^\circ\text{C}$  for the spacecraft might

liquify barium hydroxide, requiring special design considerations should it be used.

The LiOH was compared to  $\text{Ba}(\text{OH})_2$  in the Tissuglas pad form using the bent tube incubation chamber. Three separate experiments in which  $\text{Ba}(\text{OH})_2$  outweighed LiOH by about a factor of two led to the conclusion that the LiOH was more sensitive by a factor of from 1.3 to 2.2 to *E. coli* produced metabolic gases. (Stoichiometrically, for equal weights,  $\text{LiOH} \cdot 1 \text{ H}_2\text{O}$  will take up 3.95 times more  $\text{CO}_2$  than  $\text{Ba}(\text{OH})_2 \cdot 8 \text{ H}_2\text{O}$ .) Figure III-11 is typical of these curves. However, a similar run using chemical generation of  $\text{CO}_2$  (one drop per minute of  $\text{Na}_2\text{C}^{14}\text{O}_3$  solution into powdered citric acid) indicated that the two compounds are about equal in sensitivity. The difference noted when using chemical and metabolic generation might be due to the greater sensitivity of  $\text{Ba}(\text{OH})_2$  to all the metabolic gases, whereas LiOH seems to reject other metabolic gases in favor of  $\text{CO}_2$ . That is, with *E. coli* generation, the available  $\text{Ba}(\text{OH})_2$  is depleted by combining with nontagged metabolic products in addition to tagged  $\text{CO}_2$ , while the LiOH has more selectivity toward the  $\text{CO}_2$ . Greater beta absorption in the thicker (mass/area)  $\text{Ba}(\text{OH})_2$  collectors is another factor to be considered.

Reproducibility of the LiOH pads was the next characteristic investigated. This was accomplished by exposing LiOH pads in window planchets to the same tagged  $\text{CO}_2$  atmosphere and counting each with a geiger tube. Although several runs showed fair reproducibility, others showed deviations that were intolerable. In the best run of ten pads, the pad with

the highest count rate per milligram of collector was 10 percent greater than the average, and the lowest was 7.5 percent less than the average. It seems likely that the technique used to make the pads can be improved so that reproducibility will be maintained and each pad will have equal distribution of hydroxide.

#### D. NONMETABOLIC GAS REMOVAL

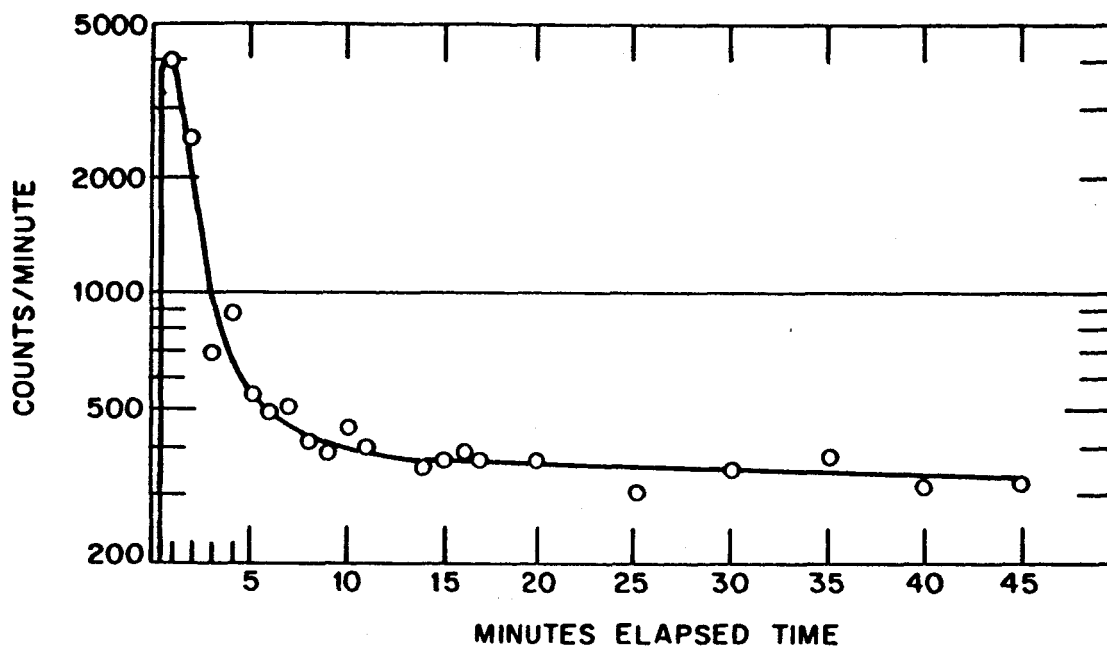
While sealed in ampules, the tagged broth breaks down to some extent to form nonmetabolic gas products which, if not removed, contribute to the background of the sterile control. Because it is desirable to reduce the sterile control level as much as possible, removal of these gases is a process of considerable importance.

Gulliver III presently incorporates devices for flushing the incubation chamber with air, but experiments in the past have cast doubt as to the effectiveness of this method, at least as to the extent carried out in the present design. Experiments in breaking ampules of broth containing dissolved tagged  $\text{CO}_2$  have indicated that there is a definite tendency for the  $\text{CO}_2$  to evolve naturally without flushing if the ampules are broken at Mars atmospheric pressure (about 0.1 earth atmosphere).

The work carried out this quarter has been directed toward improved experimental procedures to obtain more sensitive determination of the extent of this evolution at Mars pressure.

Ampules of M-8 broth, untagged and sterile, were saturated with tagged  $\text{CO}_2$  with an activity of one microcurie per milliliter. A quantitative analysis was performed to determine the solubility of  $\text{CO}_2$  in M-8 broth; the result was 1.16 ml/ml. Therefore, each three milliliter ampule contained 3.48 microcuries--assuming no losses during the loading process.

A dummy incubation chamber of the Gulliver III interior dimensions was used in the experiments. This device was loaded with ampule, string, and firing squib as in the Gulliver instrument; then placed in a vacuum desiccator and pumped down to 3.2 inches Hg absolute. The ampule was broken by firing the squib. Counting of the released gas was accomplished with an Amperex 18515 geiger tube (the type used in Gulliver III) mounted above the baffle. No gas collector was used, and the baffle was always open. As the gas came out of solution and diffused out of the string ports, its presence was determined relatively by the count rate as time elapsed. To prevent diffusion back into the incubation chamber, a leak was introduced into the desiccator and the pump kept running to provide simulation of an infinite volume as an actual open Mars atmosphere. The curve of this count rate as a function of time is given in Figure III-12. It can be seen that after about 15 minutes, the bulk of the tagged gas had evolved from the incubation chamber. This elapsed time was selected as that to use in the following experiments. These tests established the gross nature of the  $\text{CO}_2$  removal, but it was recognized as not indicating the quantity of dissolved  $\text{CO}_2$  that would be



NOTE: No gas collector was used, therefore the count rate at any time is proportional to the amount of gaseous  $\text{CO}_2$  remaining in the incubation chamber. The contamination of the chamber resulted in a post-test background of 260 cpm.

Figure III-12.  $\text{CO}_2$  evolution from dummy chamber at Mars pressure.

present after the string port closed and the baffle opened in a Gulliver III instrument.

In order to obtain a look at the possibly significant gases remaining after 15 minutes of natural diffusion, the next experiments were done using the Gulliver instrument with collector pads and sealed-off baffles in the normal manner of Gulliver operation.

Before using Gulliver III in vacuum it was necessary to develop a protective device over the face of the geiger tube so that it would not bow out and rupture under vacuum. Several failures resulted before a successful device and geiger tube were found. The device consists of a metallic annulus with cross pieces formed to the contour of the geiger face. With a soft Tisuglas collector pad in place, the mica window of the geiger is restrained by the cross pieces at reduced pressures. So far, all five runs under vacuum have been made without a tube failure.

The first run showed no activity on the collector pad when the ampule was smashed and the gas allowed to diffuse out the string ports for 15 minutes before the baffle valve to the gas collector and geiger tube was opened. The atmosphere within the desiccator was 97 percent  $N_2$ , 3 percent  $CO_2$ , maintained at 2.7 inches absolute by constant pumping and replenishing from a tank of compressed gas of this composition. (The 3 percent  $CO_2$  was selected to furnish a somewhat more severe concentration than is believed to exist on Mars, in case this factor affects adversely the solubility of the tagged  $CO_2$  in the broth.) If the results of this run were valid

experimentally, the conclusion would follow that nonmetabolic gases would present no problem if allowed to diffuse for about 15 minutes on Mars. But, unfortunately, suspicions that the ampule used in the experiment had leaked during storage were substantiated by the next two runs.

The following runs were made exactly as the first, with hopes, but not expectations, of seeing such favorable results. The curves of count rate as a function of elapsed time after the baffles were opened closely resemble the familiar sterile control curves. (See Figure III-13.) The conclusion drawn from these curves is that there may still be significant nonmetabolic gases left in the incubation chamber after 15 minutes of diffusion at Mars pressure and composition, even though the bulk of this gas has been eliminated.

Because the level to which these curves rise may not reflect the activity to be expected from naturally evolved nonmetabolic gases from the broth, the next experiment was to compare sterile control levels in simulated Mars atmosphere and in earth atmosphere. Both instruments were loaded as if for a standard field test, but no inoculation was made. The curves of this run are given in Figure III-14. It appears from these curves that the effect of Mars atmosphere on the sterile control level is a significant reduction. Since this experiment has not been repeated, it would be premature to draw a firm conclusion.

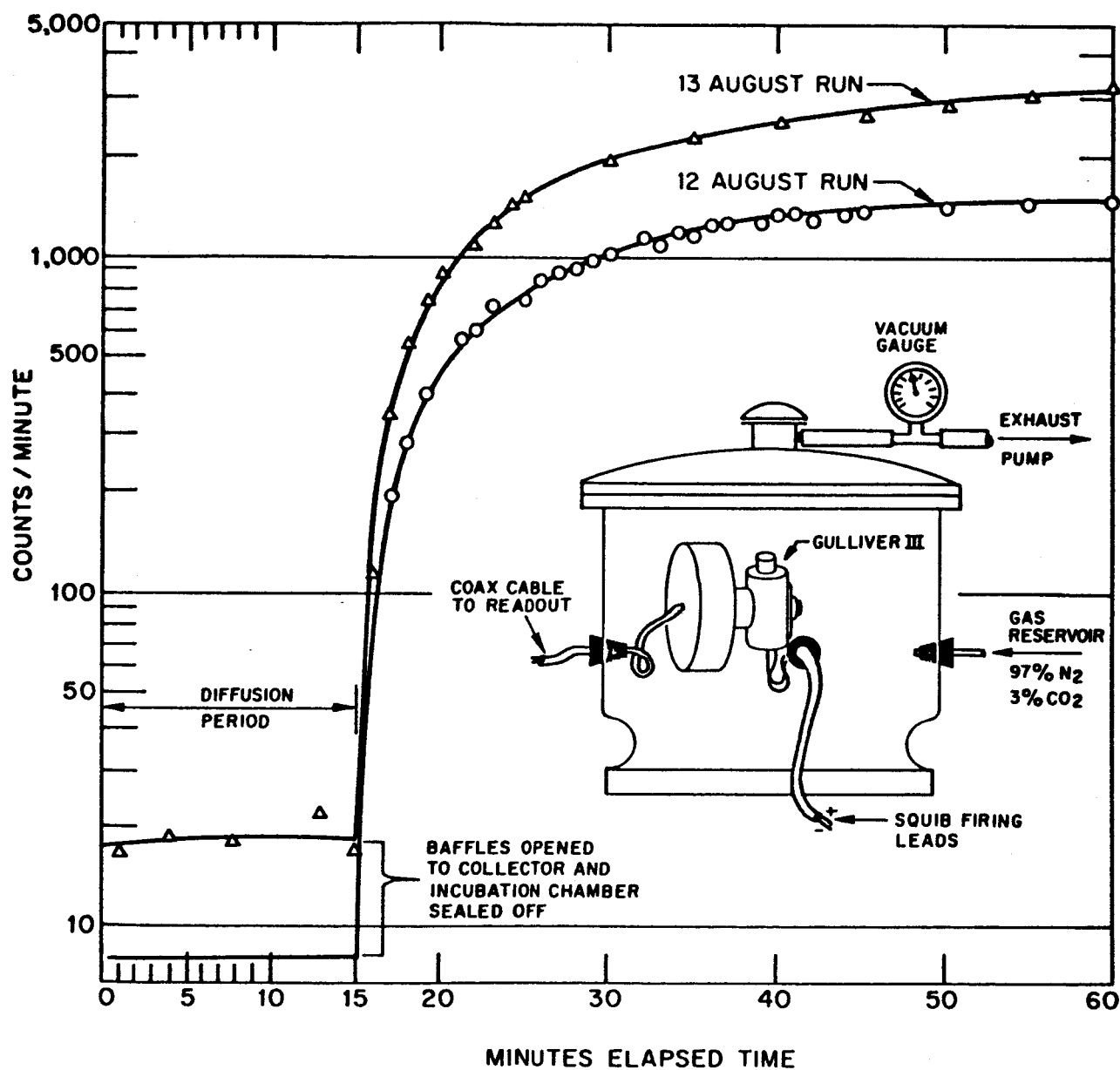
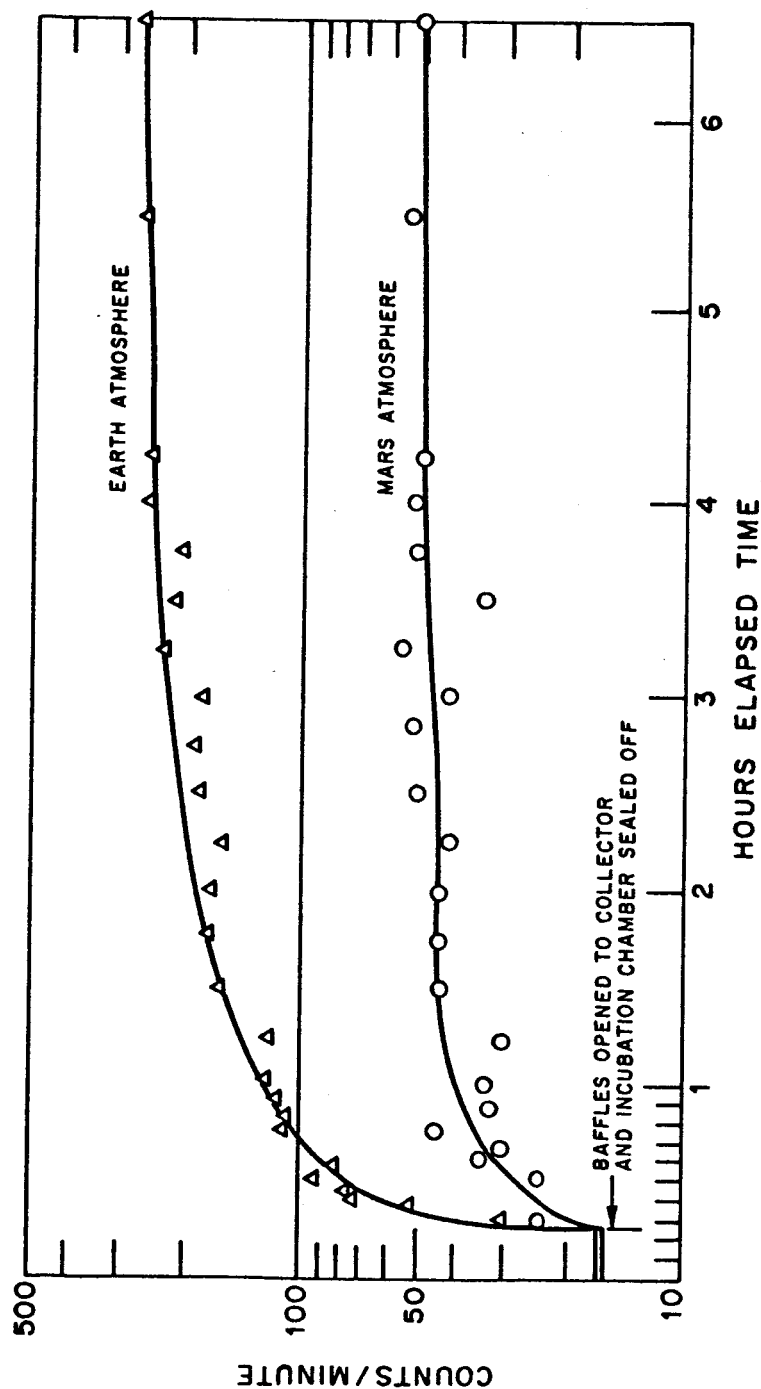


Figure III-13. Collection of residual nonmetabolic  $\text{CO}_2$  in Gulliver III after 15 minutes diffusion under simulated Mars pressure and composition.



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NOTE: The lower curve is for the unit which diffused in simulated Mars pressure and composition for 15 minutes, while the upper curve is for earth atmosphere. The Gulliver instruments were prepared and operated as in standard field tests except for soil sampling.

Figure III-14. Sterile controls for earth and Mars atmospheric pressure and composition.

E. SAMPLE COLLECTION

No experiment was performed on this phase during the quarter.

Plans are being made for work in this area very soon.